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THE EFFECT OF DIETHYLSTILBESTROL UPON ALLOXAN DIABETES IN THE MALE RAT

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DIETHYLSTILBESTROL and other estrogens are diabetogenic in partially depancreatized and normal force-fed rats (Ingle, 1941). More recently, James and Dawson (1946) reported that diethylstilbestrol failed to intensify the glycosuria of alloxan-treated rats which ate food *ad libitum*. In the present study it was shown that diethylstilbestrol caused exacerbation of alloxan diabetes in the force-fed rat but that in similar animals eating *ad libitum* the diabetogenic effect of the estrogen was either partially or completely masked by the concomitant decrease in food intake. In animals having severe alloxan diabetes the administration of diethylstilbestrol caused a striking decrease in glycosuria due to inhibition of appetite.

METHODS

Male rats of the Sprague-Dawley strain were maintained on Purina Dog Chow until they reached a weight of 310 gm. They were then fed a medium carbohydrate fluid diet made according to Table 1. During the administration of alloxan all of the rats ate the diet *ad libitum*. Alloxan was injected intraperitoneally in doses of 25 mgm. every other day until glycosuria appeared. In most of the animals two doses of alloxan were sufficient to cause some degree of diabetes, but more resistant animals were given as many as six injections. The animals were placed in metabolism cages and some of them were force-fed by stomach tube each morning (8:30 to 9:15 A.M.) and late afternoon (4:15 to 5:00 P.M.). The techniques and the diet were modifications of those described by Reinecke, Ball and Samuels (1939). During the period of adaptation to forced feeding, the amount of diet was increased gradually to prevent the development of "food-shock." The ani-

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mals were brought to a full feeding of 26 cc. (29 gm.) of diet per rat per day on the fifth day. The food for *ad libitum* eating was placed fresh each morning in clean glass jars. The amounts of food consumed each day were determined by weighing at the same time each morning. It is important to note that there was no wasting of this fluid diet by the animals. We have never been able to eliminate the wastage of dry diets. There was a minor and relatively constant error due to evaporation.

The animals were maintained in an air-conditioned room in which the temperature was maintained at 74 to 78° F. and the humidity at 30 to 35%

TABLE 1. MEDIUM CARBOHYDRATE DIET

Constituent	Grams
Cellu flour (Chicago Dietetic Supply)	120
Osborne & Mendel salt mixture	40
Diet yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Vitamin K (2-methyl-1,4-naphthoquinone)	100 mg.
Mazola oil	200
Casein (Labco)	160
Starch	200
Dextrin	190
Sucrose	200
Water to make total of	2000 cc.

of saturation. Twenty-four-hour samples of urine were collected at the same hour (8:00 to 8:30 A.M.) and were preserved with thymol. Urine glucose was determined by the method of Benedict (1911).

The diethylstilbestrol was in sesame oil solution and was given by subcutaneous injection once daily in a volume of 0.1 cc.

EXPERIMENTS AND RESULTS

During preliminary studies on alloxan diabetes in the rat, we observed a definite tendency for the level of glycosuria to decrease during the first two weeks. Rats which showed a mild glycosuria following alloxan injections frequently became free from glycosuria and some regained a near normal resistance to the exacerbation of the diabetic state by diethylstilbestrol. It can be assumed that islet tissue which was made hypofunctional by initial damage regained a part of its functional capacity by healing or by the hypertrophy of tissue. It was necessary to observe the alloxan-injected rats for periods of three to four weeks until a steady state of glycosuria was established before the tests of the estrogen were started. It was a simple matter to produce a severe state of alloxan diabetes, but it was relatively difficult to establish a slight or mild permanent glycosuria. Also, in preliminary experiments it was found that the force-fed rat having alloxan diabetes was easily killed by the too-rapid exacerbation of the diabetic state by large doses of diethylstilbestrol, just as was shown earlier (Ingle, 1941) for the partially depancreatized, force-fed rat.

In Experiment 1, 12 rats were studied which had become free from glycosuria following the initial injections of alloxan. Six rats were force-fed 26 cc. (29 gm.) and the remaining 6 ate the same diet *ad libitum*. After 14 days all of the rats were injected with 0.1 mgm. daily of diethylstilbestrol for 7 days and the dose was then increased to 1.0 mgm. for 7 days. Five of the 6 force-fed rats developed a mild glycosuria (maximum value of 2273 mgm. for 1 day) during the period of estrogen treatment, and the glycosuria disappeared when the estrogen was withdrawn. These animals gained weight at a reduced rate during the injections of the estrogen. In contrast, all of the 6 animals which ate *ad libitum* developed anorexia, lost weight rapidly, and only one rat excreted glucose (398 mgm.) for one day. These data are not summarized in the charts.

In Experiment 2, 5 rats having severe diabetes ate *ad libitum* throughout the experiment. After a period of 14 days all of the animals were given 0.1 mgm. of diethylstilbestrol daily for 7 days. They all showed a sharp reduction in food intake and lost weight rapidly. The average daily value for urinary glucose was reduced from approximately 11 gm. to slightly less than 5 gm. When the injections of the estrogen were stopped these changes were reversed. These data are not summarized in the charts.

Experiment 3 was similar to Experiment 2 and gave similar results. Five severely diabetic rats were injected with 0.1 mgm. of diethylstilbestrol daily for 7 days, the injections were stopped for 10 days and 1.0 mgm. of diethylstilbestrol was given daily for 7 days and then withdrawn. There was a sharp fall in the intake of food during periods of estrogen injection and an accompanying fall in the level of urinary glucose and a marked loss of weight. When the injections of the estrogen were stopped the changes were reversed. These data are summarized in Figure 1.

Experiment 4 was a study of 5 rats having mild alloxan diabetes. The animals were force-fed during the first phase of the experiment. During the administration of 0.01 mgm. daily of diethylstilbestrol for 7 days, there was a marked increase in the level of urinary glucose and a small loss of weight in each animal. When the injections of the estrogen were stopped these changes were reversed. Following the second control period the forced feeding was stopped and the animals were permitted to eat *ad libitum* during the remainder of the experiment. During this second phase of the experiment the injection of 0.0[1] mgm. of diethylstilbestrol daily failed to increase the severity of the glycosuria; in fact, glycosuria was abolished in all of the animals. There was a sharp decrease in the intake of food and a marked loss of weight. When the dose of diethylstilbestrol was increased to 0.1 mgm. per day, a mild glycosuria was reinduced in all 5 of the animals but was much less marked than the glycosuric response of the same animals during forced feeding. When the estrogen was withdrawn, the

intake of food was increased and the animals gained weight rapidly. All of the animals remained free from glycosuria during the final control period. These data are summarized in Figure 2.

DISCUSSION

The data of these experiments show that diethylstilbestrol has a very marked diabetogenic effect in the force-fed, alloxan-injected rat just as in the force-fed, partially depancreatized rat (Ingle, 1941). When similar animals ate *ad libitum* the diabetogenic effect of the estrogen was masked by the accompanying reduction in food intake.

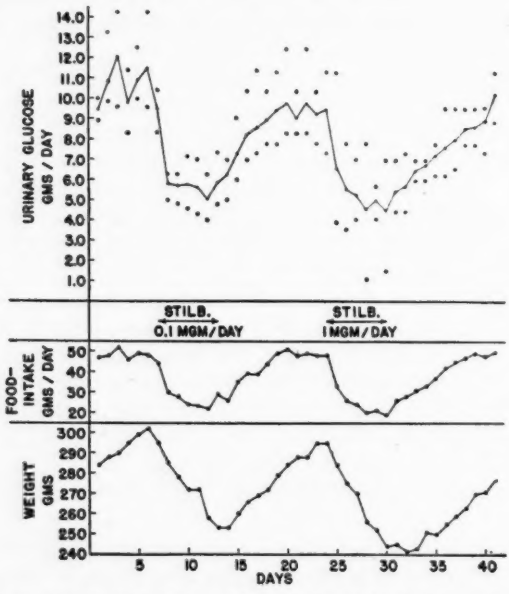

FIG. 1. The effect of diethylstilbestrol in severely diabetic rats eating *ad libitum*. Averages and individual values for glycosuria.

The effect upon appetite was so marked that the glycosuria of rats having severe alloxan diabetes was reduced during the administration of diethylstilbestrol. In the mildly diabetic rats of Experiment 4, there was some increase in urinary glucose when 0.1 mgm. per day of the estrogen was given during *ad libitum* eating. At this time the anorexia was less marked than during the initial response to the estrogen.

It is important to note that these experimental animals voluntarily ate more food during their control periods than was given by forced feeding and that the level of food intake was higher in the severely diabetic rats than in the mildly diabetic rats. This effect of diabetes upon appetite is well known.

We offer the following explanation for the failure of Janes and Dawson (1946) to demonstrate a diabetogenic effect of diethylstilbestrol in the alloxan-treated rat. First, when the intake of food is not sustained by forced feeding, diethylstilbestrol causes a reduction in food intake which masks its diabetogenic action. Janes and Dawson offered this as a possible explanation for their negative results. Second, the diabetogenic effect of estrogens is demonstrable only in mildly or

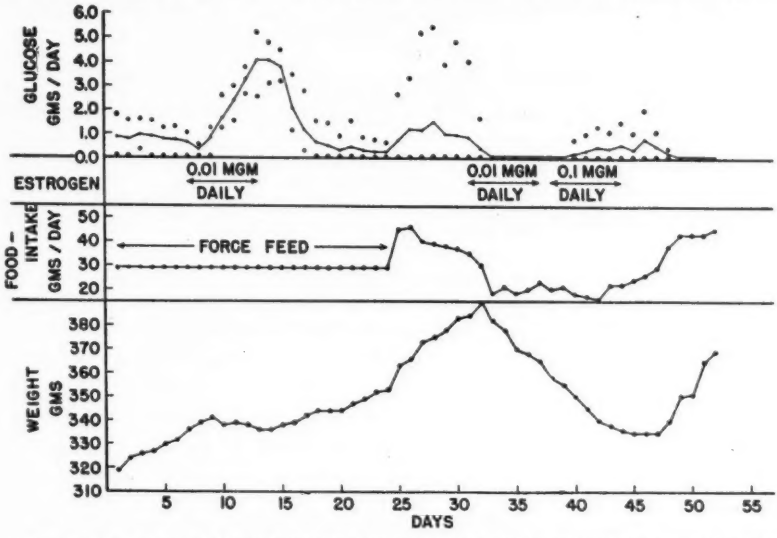

FIG. 2. The effect of diethylstilbestrol in mildly diabetic rats during forced feeding and during *ad libitum* eating. Averages and individual values for glycosuria.

moderately diabetic animals. In animals which are already totally diabetic, or nearly so, neither estrogens nor any other diabetogenic agent which we have studied can be expected to intensify the diabetes. Inspection of the data of Janes and Dawson (1946) shows that their animals were severely diabetic.

There have been a number of clinical reports to the effect that the administration of estrogens will reduce the insulin requirement of diabetic patients. Could this be explained on the basis of an effect of estrogens upon food intake? There are many reports that large doses of estrogens, especially diethylstilbestrol and chemically related synthetic compounds, cause anorexia in the patient. It is not possible to answer this question from a review of the clinical literature, for no exacting studies of food intake by such patients have been reported.

SUMMARY

This was a study of the effect of diethylstilbestrol upon alloxan diabetes in rats which were force-fed and in similar animals which ate

ad libitum. Diethylstilbestrol was found to cause exacerbation of diabetes in the force-fed rat, but in similar animals eating *ad libitum* the diabetogenic effect of the estrogen was either partly or completely masked by the concomitant decrease in food intake. In animals having severe alloxan diabetes the administration of diethylstilbestrol caused a sharp decrease in glycosuria due to a decrease in the voluntary intake of food.

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INCREASED FAT FACTOR PRODUCTION AFTER ADRENAL ENUCLEATION*

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IT IS WELL known that, in certain species of mammals, removal of part of the adrenal cortices leads to hypertrophy of the remainder. Thus, removal of one adrenal in the rat is followed by considerable hypertrophy of the other gland, according to MacKay and MacKay (1926). The effect is most striking if one enucleates both adrenals. After several weeks, regeneration occurs so that the mass of adrenal tissue may equal the original (Ingle and Higgins, 1938). Such observations fail to reveal the functional activity of the remaining tissue during the period of regeneration. In our own laboratory, experiments on the albino mouse have shown that within three weeks after enucleation of both adrenals, there is an increase in fat factor production (as indicated by lipid deposited in the liver after starvation (Brownell, 1946)) which reaches values far above normal. In the present study we have followed the production of this factor for some time after enucleation.

METHODS

Male albino mice of the Harlan strain, 10 to 14 weeks of age, were employed. These had been kept in the laboratory for one week or longer on a Purina chow diet before the operation in order to insure as little variation as possible in the nutritive condition. Both adrenals were enucleated at one operation through the lumbar route under ether anesthesia. The adrenal was held up by the suspending connective tissue and the capsule slit with an iridectomy knife. Gentle squeezing with a second pair of forceps ejected the contents. Then the capsule, containing a small amount of adherent cortex, was pushed back into place. Double adrenelectomy was performed through the same route at one operation. Adrenalectomized mice were given 1% NaCl solution as drinking water.

The assay was based on the fact that normal animals deposit fat in the liver during starvation, while adrenalectomized animals fail to do so. The amount of liver fat has been determined chemically, and estimated histologically, after 24 hours' starvation. Determinations were made on the livers of both normal and adrenalectomized mice to serve as controls. The tests were carried out on the adrenalectomized animals two days after the operation. The adrenalectomized animals were fed 24 hours and then fasted 24 hours.

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Chemical method

The livers were removed under nembutal anesthesia, weighed and ground to a pulp with sand. The pulp was thoroughly extracted with boiling 3–1 alcohol-ether mixture as suggested by Bloor (1943). After removal of the solvent, the residue was extracted with boiling petroleum ether. The petroleum ether was removed and the residue, determined gravimetrically, is reported as total liver fat.

Histological methods

Blocks of liver tissue from corresponding lobes were fixed in 10% formalin, sectioned by the freezing method and stained with Sudan Orange. The sections were cut at 10 micra with a precision microtome to insure uniform thickness. In estimating the amount of Sudanophil lipid, the following grading system was employed: 0 = no stainable lipid (according to chemical analysis, however, these tissues contained 5–6% lipid); 1 = small amount of stainable lipid in cells widely scattered in the lobules; 2 = an increased amount of lipid localized in a narrow zone either at the periphery of the lobule or about the central vein; 3 = lipid laden area increased to include approximately one-half of the entire lobule; 4 = lipid area increased to approximately three-fourths of the lobule area; 5 = lipid laden area extended to include the entire extent of each lobule; 6 = more densely packed lipid than 5. Typical sections of each of these grades were selected as standards. In practice it was found possible to recognize finer degrees of distinction. Consequently, + and − were also employed. Thus, if a given section, when compared with the standard sections, was found to contain slightly more lipid than a standard 2, it was graded 2+. But if it resembled the standard 3 more than 2, it was graded 3−. For the quantitative evaluation of these histochemical findings the arithmetical mean of the various grades of Sudanophil fat was computed. One individual made all of the evaluations without knowledge of the values by the chemical method.

Adrenals were weighed by means of a Roller-Smith torsion balance. All enucleated adrenals were fixed with formol-Zenker and sectioned, in order to ascertain the amount of regeneration.

RESULTS

Liver fat

Chemical determination

Except for the mice tested at 2 days and at 6 months after enucleation, the number of animals used on a point was large enough to indicate the range of variation.

The amount of fat found in the livers of 12 normal mice after 24 hour starvation was $8.51 \pm 1.25\%$ (Fig. 1) while that in 29 adrenalectomized mice was $6.31 \pm 0.71\%$. Two days after enucleation, the value of 6.64 ± 2.43 from seven animals, although from too small a sample to be significant, indicated no increase above the adrenalectomy level. However, at seven days in 13 mice, the liver fat had risen to $12.23 \pm 3.4\%$ which, when compared with the value for normal mice, gave a t value of 3.55, which is significant. At 15 days in 11 mice,

the liver fat was $9.97 \pm 1.9\%$, which gave a t value of 3.18 when compared with that for normal mice, which is insignificant.

The next determinations were made on the livers of 21 animals 99 to 118 days after enucleation. The value obtained was $9.97 \pm 2.74\%$ which, compared with that for normal animals, gave 3.0 for the value of t and is, therefore, significant. The liver fat was determined in 5

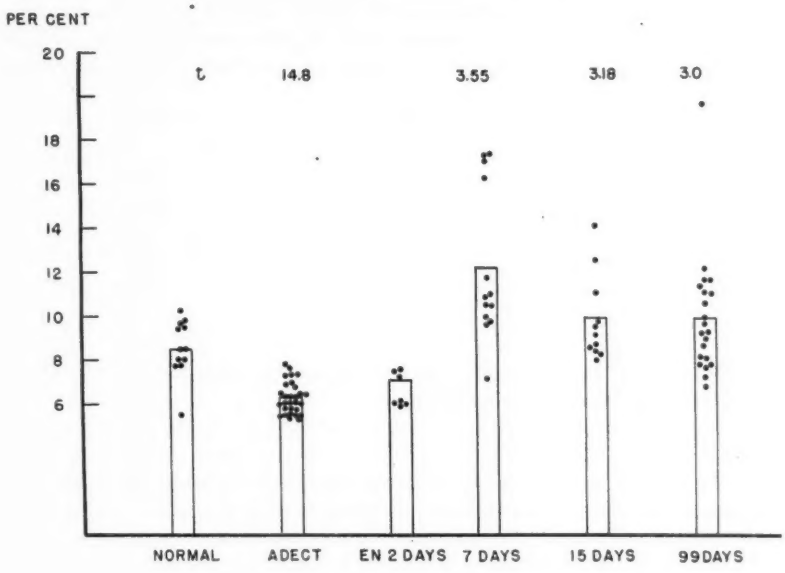

FIG. 1

mice 300 days after enucleation. The values ranged from 6.92% in an animal in which one adrenal had disappeared, to 9.95% in that with the highest value; the other three values were 7.71, 8.61 and 9.85%. These are within the normal range.

Histological estimate

The range of values estimated from histological sections for the fat in the livers of normal animals lay between 0+ and 5, with an average of 2+. The range for the fat from the livers of adrenalectomized mice was from 0 to 2+ with an average of 1, while for 2 days enucleation the range was 0+ to 1+, with an average of about 1. The range for 7 days enucleation was 3 to 6 with an average of 5. The range for 15 days enucleation was 2+ to 5− with an average of 3+. The range for 99 to 118 days enucleation was 2− to 6+ with an average of 3+.

A comparison of the mean histological values with the chemical determinations is shown in Table I. Although not quantitative, the

histological evaluation can give relative estimates of lipid in the range between the adrenalectomy level and marked hypersecretion, although it is less dependable in the very high values. It should be noted also that at the low histological rating of 0+ to 1 there is still 5 to 6% of lipid present. This is largely invisible lipid.

From these observations, it appears that within a week after enucleation of both adrenals, the output of fat factor from adrenal tissue reaches its maximum which is much higher than normal. In the few instances where it is not excessive, this may be accounted for by too great destruction of adrenal tissue. The production of fat factor falls

TABLE I. COMPARISON OF HISTOLOGICAL EVALUATION WITH CHEMICAL DETERMINATIONS OF LIVER LIPID

	Per cent by chemical analysis	Histological evaluation
Normal	8.5	2+
Adrenalectomized	6.3	1
Enucleated— 7 days	12.2	5
Enucleated—15 days	10.0	3+
Enucleated—99 days	10.0	3+

soon after to a lower value, which remains significantly higher than normal for at least three months.

Regeneration

The amount of adrenal tissue available for hormone production at different stages after enucleation is indicated by the following observations.

The adrenals of ten male mice were removed, weighed and then enucleated in the usual manner. The remnants of the two adrenals left after enucleation ranged from 18 to 30% of the original glands, with an average of 24% by weight. This includes the capsule, so that it is not all active tissue. Moreover, it is unlikely that all of the active tissue would survive because of the disturbed circulation.

At *2 days* after operation, if the enucleation was thorough, a mere shell, consisting of a few layers of live cells, persisted, with a central mass of coagulum and debris. Occasionally, one almost complete adrenal remained, due to a failure to remove the capsular contents. But because of the incident disruption the output of fat factor was at the "adrenalectomy" level.

Seven days after enucleation of both adrenals in eight mice, the glands weighed an average of 0.0136% of the body weight. The adrenals from 100 normal male mice of the same strain weighed $0.0133 \pm 0.0030\%$ of the body weight. However, these values are misleading since histological examination showed that, in many cases, a considerable portion of the adrenal mass was hemorrhagic coagulum. In four of these the adrenal was completely destroyed on one side yet the

liver lipid was high (9.8, 10.5, 10.9 and 17.0%), the highest value occurring in an animal which possessed a very small amount of cortical tissue. In five other mice, in which much of the adrenal tissue had been destroyed on both sides, all but one gave high liver lipid (7.2, 9.7, 10.0, 10.5 and 11.8%). In the four remaining animals, only one adrenal was destroyed extensively, while a considerable portion of the other remained. Of these, three gave very high liver lipid while one gave a high value (17.4, 17.2, 16.2 and 10.9%).

It is very evident from these observations that a relatively small mass of cortical cells is capable of producing a relatively large amount of hormone.

Fifteen days after enucleation the amount of cortical tissue appeared to have increased somewhat according to the histological picture, but decreased decidedly in weight due to disappearance of inactive material. At this time the regenerated adrenals of 11 mice weighed an average of 0.0069% of the body weight, which is about half of the weight of normal adrenals. The range was great, from 0.0020 to 0.0115% of the body. In six instances, only one adrenal remained, the other apparently having been absorbed. The liver lipid values were within the normal range except for three animals.

At 99 to 118 days. The adrenal tissue from 15 animals was studied histologically at this stage of regeneration. In 7 mice, adrenal tissue was absent on one side. On the other side, the regenerated mass was small. In 8 mice, small adrenals composed only of cortical tissue were found on both sides. In none of the whole group had an adrenal regenerated to apparently normal size. Moreover, the liver lipid was no higher in those animals in which there was more adrenal tissue, such as in those with two adrenals. The highest value, 19.6, occurred in a mouse with only one adrenal. It appears, therefore, that the adrenal tissue had not regenerated sufficiently to equal the amount of tissue removed. However, this reduced tissue was continuing to produce more than the normal amount of hormone.

At 300 days. Of five mice, two possessed only one adrenal, but that was well developed, i.e. cortex appeared as large as normal. Two of the others possessed adrenals with cortices about normal size, while the third possessed one small adrenal and one of normal size.

DISCUSSION

From our evidence it is concluded that the histological method gives a valid indication of the relative amount of visible lipid in the liver but not of the absolute amount. For that, the chemical method is necessary. From the fact that little or no visible lipid appears in the liver of the starved adrenalectomized mouse, it seems that the histological method gives a true indication of the lipid under the control of the adrenal. Deducting the lipid found by chemical determination in the liver of the adrenalectomized animal (6%) from the values found

in other conditions, the adrenal controlled lipid is as follows: normal mouse, 2.5%; mouse with adrenals enucleated 7 days, 6.2%; mouse with adrenals enucleated 15 to 118 days, 4%.

We were surprised to find that the output of fat factor exceeded the normal amount after enucleation, but more remarkable was the fact that the maximum was reached while the amount of cortical tissue was still near the minimum. This is proof that the cortical cells have great capacity for secretion which can be sustained for a considerable time, since the hypersecretion is maintained for weeks while regeneration is occurring.

The sudden reduction of hormone production by enucleation would remove an inhibiting effect on the anterior pituitary so that it soon would produce more than the usual quantity of adrenocorticotrophic hormone. The cortical remnants would respond under the increased stimulation. However, the increased output of fat factor by the adrenal cortex apparently does not, for some time, cause the adrenocorticotrophic hormone production to return to the normal level. It is difficult to understand this readjustment to a higher level.

Evidence has been obtained by Li and P'An (1940) of an increase in adrenocorticotrophic hormone production after removal of both adrenals of a rat. Such an animal was connected parabiotically with a second animal. The adrenals of the latter became enlarged.

This is not the first time that increased activity of the adrenal cortex has been shown following removal of part of the cortical tissue. It was shown several years ago (Lockwood and Hartman, 1933) that following the removal of one adrenal in the guinea pig, there was overactivity of the remaining adrenal for about three weeks following the operation. This was indicated by the protection afforded against the onset of scurvy, similar to that given by adrenal extract injections. A like increase in activity after removal of one adrenal was indicated in the rat, also in thiamin deficiency.

Removal of the inhibitory influence on the pituitary by ovariectomy results in an increase in the glyconeogenetic power in the rat beginning about 70 days after the operation (D. Smith, 1946). Removal of part of the adrenal cortex by demedullation reduced the ability of rats to deposit glycogen in the liver after fasting for 24 hours at one-half an atmosphere. This was so 10 to 18 days after demedullation (G. Evans, 1934). In the mouse, we have found an increase in the output of the glyconeogenetic factor at a later time after enucleation of the adrenals than that for the fat factor (unpublished work).

SUMMARY

The amount of liver lipid in starved mice was determined chemically in normal and adrenalectomized animals and in animals whose adrenals had been enucleated previously. It was also estimated histologically.

It appears that the visible lipid is under the control of the adrenal. The balance shown by chemical determination may be bound lipid.

Within a week after enucleation of both adrenals, the output of fat factor reaches its maximum, which is much higher than normal. The production of fat factor falls soon after to a lower value, which remains significantly higher than normal for at least three months. Our observations demonstrate that a relatively small mass of cortical cells can produce a relatively large amount of fat factor and that it can maintain this hyperactivity for weeks.

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INTRACELLULAR COLLOID DROPLETS AS A BASIS FOR THYROTROPHIC HORMONE ASSAY IN THE CHICK[1]

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FORMATION of intracellular colloid droplets in the thyroid epithelium occurs soon after the administration of anterior pituitary gland substance in a variety of species (Grant, 1930; Grant, 1931; Severinghaus, 1933; Uhlenhuth, 1934; Okkels, 1934; Ponse, 1938; Aleschin, 1935, 1939). In 1942 DeRobertis suggested that the number of droplets formed be used in thyrotrophic hormone assay. In 1944 DeRobertis and DelConte described a procedure using guinea pigs. This assay method was extremely sensitive, being able to detect as little as 0.0002 Junkmann-Schoeller (J.S.) units. Colloid droplets were preserved through use of the freezing-drying technique of Altmann-Gersh (Gersh, 1932).

It seemed of interest to attempt a thyrotrophic hormone assay based on the formation of intracellular colloid droplets in the chick thyroid, since this gland has been found sensitive and useful in assays based on increase in weight (Smelser, 1938; Kabac and Liapin, 1938; Cope, 1938; Bergman and Turner, 1939), or on increase in epithelial cell height (Rawson and Salter, 1940), or on decrease in iodine concentration (Stimmel, McCullagh and Picha, 1936). A comparison of the sensitivity of the changes in gland weight, cell height and colloid droplets also would seem of interest. Fraenkel-Conrat, Fraenkel-Conrat, Simpson and Evans (1940) and Albert, Rawson, Merril, Lennon and Riddell (1946) have compared the responses of chick thyroid weight, cell height and iodine concentration after thyrotrophic hormone administration.

It also seemed desirable to use a fixative that would preserve the intracellular colloid droplets in the thyroid gland without a freezing-drying apparatus. This would make an assay based on these droplets more widely available, and should provide a technique useful in other studies of intracellular colloid.

MATERIALS AND METHODS

Day-old White Leghorn cockerels[3] were used. They were housed in a brooder kept at about 30° C.

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[1] Aided by a grant, administered by Dr. P. E. Smith, from the Rockefeller Foundation.

[2] Assistant Resident in Medicine, Presbyterian Hospital, N.Y.C.

[3] Obtained from Hall Brothers Hatchery, Inc., Wallingford, Conn.

The thyrotrophic hormone used was a five year-old flavianic acid precipitate prepared from whole hog pituitary glands by Dr. George Smelser according to his method (1938).[4] The powder was dissolved in distilled water with the aid of the smallest necessary amount of solid sodium carbonate.

The potency of the powder was determined by assaying it for its effect on thyroid gland weight and cell height. A volume of 0.2 cc., containing 100 μg. or 200 μg. or 400 μg. of the powder, was injected subcutaneously each day for four days, beginning on the first day after hatching. Ten chicks were used at each dosage. Autopsy was performed on the fifth day, twenty-four hours after the last injection. Ten untreated chicks served as controls.

The time after a single subcutaneous injection at which the maximal number of droplets were present and the thyroid epithelium reached the greatest height was determined by injecting 50 μg. of the powder in 0.5 cc. of water. Groups of ten or twenty chicks, 3 days old, were sacrificed one, two, two and one-half, three, four, six, twelve and twenty-four hours after the injection. A maximum number of droplets were present two hours after the injection.

The effect of various dosages on the number of droplets was determined in groups of twenty chicks, three days old, injected subcutaneously with 0.5 cc. containing 5 μg., 10 μg., 25 μg., 50 μg., or 100 μg. of the powder. The animals were autopsied two hours after the injection.

Several fixatives were tested for their ability to preserve the intracellular colloid droplets. Since Gutknecht (1885) and Reinbach (1894) had used alcohol fixations to demonstrate intracellular colloid, and since Langendorff (1889) and DeRobertis (1941) reported that absolute alcohol denatured colloid, it was decided to test some alcohol-containing fixatives. P.A.F. (picric acid, alcohol and formalin) and Bouin's fluid, made with seventy per cent alcohol instead of water, preserved the intracellular colloid, but cell and colloid shrinkage was marked, and the histological appearance was unsatisfactory. Carnoy's fluid resulted in the best histological fixation, with the least shrinkage of cells and colloid, and with excellent preservation of numerous intracellular colloid droplets.

At autopsy the thyroids were carefully dissected free under a binocular microscope, and weighed to the nearest 0.1 mg. The glands were then immediately fixed in Carnoy's fixative for one hour at room temperature, and rapidly embedded in paraffin. Sections at 4 μ were made and stained by DeRobertis' (1941) modification of Heidenhain's Azan stain. However, the sections were allowed to remain in the phosphotungstic acid solution for only one hour.

Cell height of the thyroid epithelium of each animal was determined with an ocular micrometer by averaging the height of a cell in each of twenty-five successive cross sections of follicles in a section through the midportion of the gland.

The number of intracellular colloid droplets of the thyroid epithelium of each animal was determined by totalling the number of large clearly visible droplets in the entire section thickness of 25 successive cross sections

[4] A generous supply of this powder was available through the kindness of Dr. Smelser.

of follicles in a section through the midportion of the gland. The nucleoli, which occasionally stain like colloid droplets, were not counted. A 10× ocular and a 1.3 mm. oil objective lens was used in counting droplets and in measuring cell heights. These measurements were facilitated through the use of an orange filter made by adding some Orange G to water, and placing the solution between the light source and the microscope.

The mean and standard deviation of the weights, cell heights and colloid droplets of the thyroids of each group, and P values comparing the means of different groups, were calculated (Fisher, 1944).

RESULTS

1. Assay based on thyroid weight

As can be seen in Table I, a progressive weight increase of the thyroid gland resulted from increasing dosage of hog pituitary extract. A chick weight unit, as defined by Bergman and Turner (1939), is the

TABLE 1. WEIGHT AND CELL HEIGHT OF THYROID GLAND OF DAY-OLD CHICKS INJECTED DAILY FOR 4 DAYS WITH A SOLUTION OF HOG PITUITARY GLAND EXTRACT, AND AUTOPSIED ONE DAY AFTER THE LAST INJECTION

Dosage—μg.		No. of animals	Thyroid gland weight—mg.				Cell height—microns			
Daily	Total		Average ±σ	P^1	P^2	Ratio control =1.00	Average ±σ	P^1	P^2	Ratio control =1.00
0	0	10	3.52±0.84			1.00	3.96±0.41			1.00
100	400	10	4.57±0.73	<0.01		1.30	4.95±0.42	<0.01		1.23
200	800	10	5.39±1.38	<0.01	0.1	1.53	5.04±0.35	<0.01	>0.6	1.27
400	1600	10	6.07±1.73	<0.01	>0.3	1.72	5.38±0.69	<0.01	0.2	1.36

[1] *P* values are those comparing the group with untreated controls.
[2] *P* values are those comparing the group with the one treated with the next lower dosage.

total amount of material which increases gland weight by fifty per cent. This is equivalent to about 760 μg. of the powder used in this experiment.

2. Assay based on thyroid epithelium cell height

A total dosage of 400 μg. of pituitary extract given over a four day period resulted in an average increase in cell height of about one micron (Table 1) or a twenty-three per cent increase over the controls. Higher dosages increased cell height somewhat more.

It is difficult to compare strictly these results with those of Rawson and Salter (1940) since they used formalin[5] or Zenker's fixation, and obtained lower control cell heights than in our chicks. Also, they injected for five days instead of four days. Nevertheless, in their study a twenty-three per cent increase in cell height was produced by one-eighth J.S. unit daily for 5 days, a total dosage of five-eighths J.S. units. Based on this crude comparison, one J.S. unit would equal about 640 μg. of our hog pituitary extract.

[5] Shrinkage in cell height is known to follow formalin fixation (Wilcke, 1935; Mayer, 1947). Different breeds of chicks may have different cell heights (Adams and Jensen, 1944).

3. Assay based on intracellular colloid droplets. a. Effect of time elapsing after injection

A normal 3 day-old chick thyroid has only an occasional large colloid droplet (Table 2, Fig. 1), and but rarely contains numerous fine blue staining granules in some thyroid cells. A single injection of 50 μg. of pituitary extract produced a marked increase in the number of large intracellular colloid droplets as early as one hour after injection. The maximal number of droplets appeared at two (Fig. 2) to two and one-half hours after injection, and rapidly declined. At 12 hours after a single injection the gland had returned to its pre-injection state with regard to the number of droplets (Table 2). These results with the chick are similar to those of Aleschin (1939) with the guinea pig and DeRobertis (1942) with the rat. However, 24 hours after injection the

TABLE 2. INTRACELLULAR COLLOID DROPLETS AND CELL HEIGHT OF THYROID EPITHELIUM OF 3 DAY-OLD CHICKS INJECTED SUBCUTANEOUSLY WITH 0.5 CC. OF A SOLUTION CONTAINING 50 μg. OF HOG PITUITARY EXTRACT (0.066 CHICK W'T. UNITS) AND AUTOPSIED AFTER INDICATED INTERVALS

Time—hrs. after injection	No. of animals	Total No. of colloid droplets in 25 follicles				Cell height—microns			
		Average ±σ	P^1	P^2	Ratio control =1.00	Average ±σ	P^1	P^2	Ratio control =1.00
0	20	3.4± 2.4			1.00	3.74±0.25			1.00
1	10	92.9±69.5	<0.01		27.32	3.96±0.42	>0.5		1.06
2	20	182.5±87.4	<0.01	<0.01	53.68	4.15±0.28	<0.01	0.2	1.11
2.5	10	180.6±63.5	<0.01	>0.9	53.12	4.27±0.49	<0.01	0.4	1.14
3	10	139.8±51.3	<0.01	0.2	41.12	4.23±0.49	<0.01	0.8	1.13
4	10	109.5±68.8	<0.01	0.3	32.21	4.34±0.28	<0.01	0.6	1.16
6	10	46.1±51.8	<0.01	<0.05	13.56	4.58±0.24	<0.01	0.05	1.23
12	10	4.9± 3.7	0.2	<0.05	1.44	4.56±0.23	<0.01	0.8	1.22
24	10	numerous small granules				4.12±0.23	<0.01	<0.01	1.10

[1] P values are those comparing the group with untreated controls.
[2] P values are those comparing the group with the one treated for the next shorter time.

epithelium was found to contain very numerous small blue-staining granules in an apical position (Fig. 3). These were particularly abundant 24 hours after the last injection in the thyroids of the animals which had been treated for four days with pituitary extract and used in the assay based on gland weight (Fig. 4).

The increase in the number of droplets and their subsequent disappearance did not exactly parallel the increase in cell height. After a single injection of 50 μg. of pituitary extract (Table 2) there was a significant increase in height as early as two hours after the injection, but it did not reach a maximum until 6 hours had elapsed. It was maintained up to 12 hours after injection. At 24 hours after injection the cell height declined significantly, but was still higher than normal (Table 2). The prompt increase in cell height of thyroid epithelium following pituitary extract administration confirms the work of Aron (1930), Krogh and Okkels (1933), Kummer (1938), Starr and Metcoff (1941), DeRobertis (1942) and Borell (1945) in guinea pigs and rats.

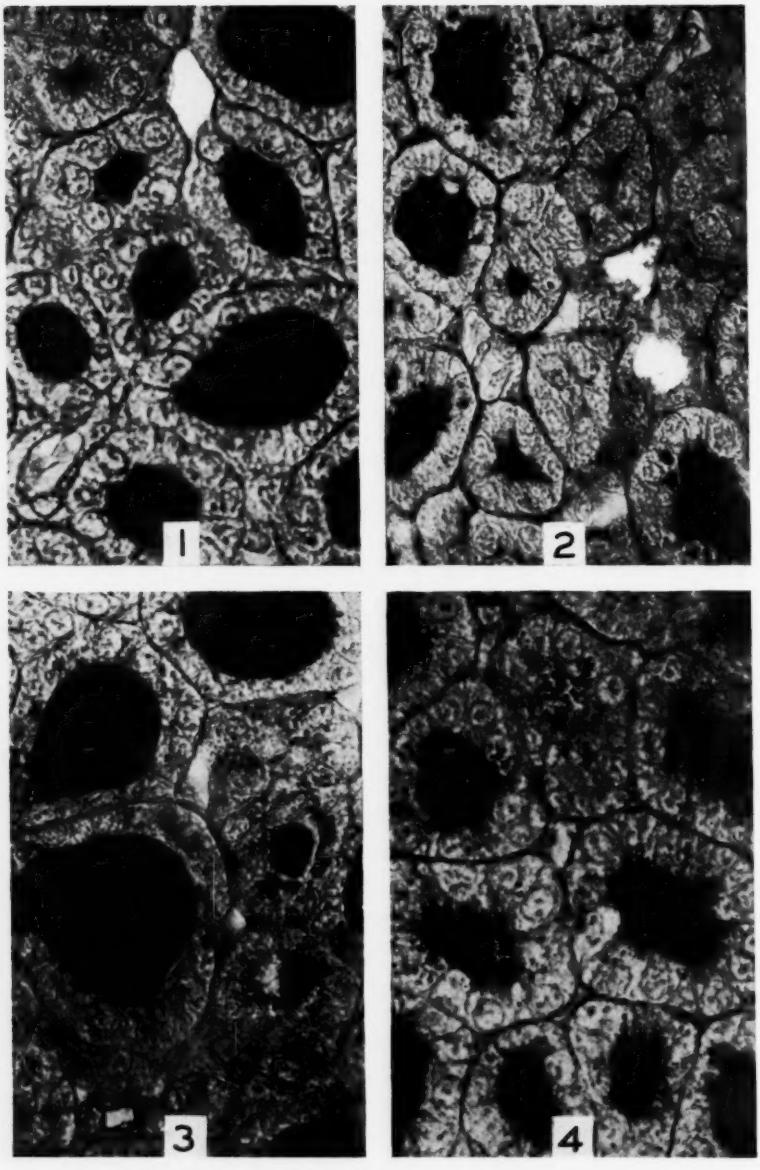

PLATE 1. Sections through the thyroid glands of male chicks. ×1040.

FIG. 1. Untreated control, 3 days old.

FIG. 2. Three day-old chick injected subcutaneously with 50 μg. hog pituitary powder (0.066 CK. Wt. U.), and autopsied two hours after the injection.

FIG. 3. Treated as chick in Fig. 2, but autopsied twenty-four hours after the injection.

FIG. 4. Five day-old chick injected subcutaneously each day for four days with 100 μg. hog pituitary powder, and autopsied twenty-four hours after the last injection.

b. Effect of various dosages, two hours after injection

A straight line may be obtained from the data in Table 3 by plotting the dosage of pituitary extract on a logarithm scale against the average total number of intracellular colloid droplets. As little as 10 μg. of the powder (equivalent to 0.0133 Chick Weight Units) significantly increased the average number of droplets, resulting in more than a four-fold increase in their number. These droplets were distributed throughout the glands, and were not restricted to the central follicles, as had been reported with low dosages in the guinea pig by DeRobertis and DelConte (1944). A dosage of 100 μg. caused the average number of droplets to increase seventy-six fold. Statistically significant differences were found between the means of the groups treated with progressively increasing dosages of pituitary extract. However, great variations in response of individual animals to identical dosage was encountered (Table 3), and there was considerable overlap of values between groups.

The cell height of the thyroid epithelium increased above normal,

TABLE 3. INTRACELLULAR COLLOID DROPLETS AND CELL HEIGHT OF THYROID EPITHELIUM OF 3 DAY-OLD CHICKS INJECTED SUBCUTANEOUSLY WITH A SOLUTION OF HOG PITUITARY GLAND EXTRACT AND AUTOPSIED TWO HOURS AFTER THE INJECTION

Dosage		No. of animals	Total No. of colloid droplets in 25 follicles			Cell height—microns		
μg. of hog pituitary extract	CK. wt.[1] Units		Average ±σ	P[2]	Ratio control =1.00	Average ±σ	P[2]	Ratio control =1.00
0	0.0000	20	3.4± 2.4		1.00	3.74±0.25		1.00
5	0.0066	20	5.5± 3.9	>0.02	1.65	3.64±0.23	>0.1	0.97
10	0.0133	20	14.4± 9.8	<0.01	4.24	3.73±0.29	>0.2	1.00
25	0.0333	20	78.7± 71.7	<0.01	23.15	3.84±0.15	>0.2	1.03
50	0.0666	20	182.5± 87.4	<0.01	53.68	4.15±0.28	<0.01	1.11
100	0.1333	20	258.4±110.9	<0.02	76.00	4.35±0.46	>0.1	1.16

[1] One Chick Weight Unit is contained in 760 μg. of the hog pituitary powder.
[2] *P* values are those comparing the group with the one treated with the next lower dosage.

two hours after a single injection, in the animals treated with 50 μg. or 100 μg. of pituitary extract (Table 3). Smaller dosages failed to increase cell height within this period.

It is difficult to compare the sensitivity of the intracellular colloid droplets technique in the chick with that reported by DeRobertis and DelConte (1944) in the guinea pig. For one thing, a histological fixative was used in these experiments with the chick. This possibly failed to preserve all of the intracellular colloid, particularly since intracytoplasmic vacuoles were frequently noted in stimulated glands among the abundant intracellular colloid droplets.[6] Further, the

[6] These vacuoles were not due to the presence of fat in the epithelium, since none were demonstrable by Zwemer's (1933) technique in sections stained with Oil Red O. Fat staining droplets have been demonstrated in thyroid epithelium after large dosages of pituitary preparations containing thyrotrophic hormone (Dobyns, 1946).

guinea pig assay is reported in terms of J.S. units, and such a unit was not directly determined for our extract.

However, using the calculation presented under Paragraph 3 of Results where one J.S. unit is equal to about 640 μg. of our extract, the chick appears to be less sensitive than the guinea pig. According to these calculations, the chick test can detect 10 μg., or 0.0156 J.S. units, whereas the guinea pig test can detect 0.0002 J.S. units (DeRobertis and DelConte, 1944).

However, if one accepts the findings of Fraenkel-Conrat, Fraenkel-Conrat, Simpson and Evans (1940), a guinea pig histological unit (J.S. unit) contains about five times as much thyrotrophic hormone as a chick weight or histological unit. On this basis, a J.S. unit would be contained in about 2000 μg. of our extract. In this case, since we can detect 10 μg. or about 0.005 J.S. unit, the chick would compare more favorably with the guinea pig, but would still appear to be less sensitive.

Since the validity of these comparisons is open to serious question because of the indirectness of their derivation, strict comparison had best be delayed pending a comparison of chick and guinea pig treated with identical preparations, and studied after an identical histological technique.

DISCUSSION

A wide variety of histological fixatives had been used earlier to demonstrate the intracellular colloid. Fixatives containing osmium such as Champy's or Flemming's fluid have frequently been used (Langendorff, 1889; Hurthle, 1894; Andersson, 1894; Grant, 1931; Severinghaus, 1933; Uhlenhuth, 1934; v. Hagen, 1936). Mercury containing fixatives such as Zenker's or Susa's fluids have also been found useful (Hurthle, 1894; Bensley, 1916; Eggert, 1934; Aleschin, 1935; Aleschin, 1939; Bargmann, 1939). However, Grant (1931) found Zenker's and Helly's fluids unsatisfactory in the salamander. Bouin's fluid was able to preserve intracellular colloid in the turtle (Florentin, 1926) and in the chick and rat (Dvoskin, 1947). These fixatives, as well as Carnoy's used in this experiment, result in the formation of non-staining intracellular vacuoles in the stimulated gland, though some of the intracellular colloid is preserved and stained. Whether all these vacuoles represent colloid droplets, as is maintained by DeRobertis (1941), or whether they represent fixation artifacts, especially in epithelium where histological fixatives do preserve large numbers of colloid droplets, will only be ascertained by direct comparisons of these structures in thyroids from single animals fixed in different ways.

A review of the results of this experiment reveals that the enumeration of the number of intracellular colloid droplets permits the detection of small amounts of thyrotrophic hormone. The method suffers chiefly from variability in response of individual chicks to similar dosages. This can be partly compensated for by the use of

large numbers of chicks, at least twenty per dosage. This number is recommended by Bergman and Turner (1939) for assays based on chick thyroid weight, since this response is also subject to large individual variations. Intravenous or intracardiac injection might reduce the variability, although great variability of response is encountered in the guinea pigs receiving intracardiac injections of thyrotrophic hormone (DeRobertis and DelConte, 1944). The minimum of four animals per group suggested by these authors is obviously an underestimation of the number necessary as can be seen by examining the range of response in their animals.

That the intracellular colloid droplets appear in several species after administration of material containing thyrotrophic hormone, prepared from pituitary glands in various ways, is partial evidence for the specificity of this response. Further, the intensity of response is proportional to the dosage administered. They also appear after partial thyroidectomy (Hurthle, 1894; Tschassovnikoff, 1915; Satwornitzkaja and Simnitzky, 1932), and after the administration of thiourea (Thomas, 1944; Grasso, 1946). Also, intracellular colloid droplets form in thyroid in vitro when the gland is exposed to thyrotrophic hormone (Junquiera, 1947).

However, we have evidence (Dvoskin, 1947), that these droplets also form in vitro in the absence of added thyrotrophic hormone. Further, the thyroid can form colloid in the absence of the pituitary gland, as shown by the reaccumulation of colloid in the colloid-depleted gland of the sulfaguanidine-fed rat following hypophysectomy (Astwood, Sullivan, Bissell and Tyslowitz, 1943). Whether this is accompanied by the formation of intracellular colloid is not known. Also, no extensive survey, other than that by Galeoti (1897), has ever been made for evidence of activity in substances other than that in crude pituitary extracts containing thyrotrophic hormone. Andersson (1894) demonstrated intracellular colloid after pilocarpine injections, and Hurthle (1894) found them after bile duct ligation. The effect of various materials has not been studied in hypophysectomized animals in order to rule out the possibility of participation of the animals own pituitary.[7] The uncertainty, at present, as to the exact significance of intracellular colloid droplets detracts somewhat from their specificity for thyrotrophic hormone assay. This will remain so until more is learned concerning all conditions under which they may form.

SUMMARY

The three-day old White Leghorn cockerel was tested for its suitability for assay of thyrotrophic hormone based on the formation of

[7] We have been able to demonstrate numerous intracellular colloid droplets in the thyroid gland of the hypophysectomized adult male rat after the injection of the hog pituitary powder used in this experiment. No droplets were found in the thyroid glands of hypophysectomized littermate controls.

intracellular colloid droplets. The thyroids were fixed with Carnoy's fluid, and stained with the modified Azan technique.

With this technique it was possible to detect one-fourtieth of the amount of pituitary gland extract needed to increase cell height by one micron, an increase of twenty-three per cent, and one seventy-sixth of the amount needed to increase thyroid gland weight by fifty per cent. Progressively increasing dosages, up to ten times the minimal effective dosage, resulted in a straight line dose-response curve when plotted on semilogarithmic paper. There was great variability in animal response at each dosage level and considerable overlap of response, though the averages were significantly different when twenty chicks were used in each group.

The formation of large intracellular colloid droplets was prompt, and maximal increases occurred two to two and one-half hours after subcutaneous injection of pituitary extract. The epithelium returned to a near normal state by twelve hours after injection. Small apical granules appeared in large numbers in the epithelium twenty-four hours after a single injection, and more prominently twenty-four hours after the last of four injections.

Cell height was significantly increased two hours after the injection of pituitary gland extract, reached a peak after six to twelve hours and fell towards normal 24 hours after injection.

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ROLE OF THE ADRENAL CORTEX AND THE THYROID IN THE MOBILIZATION OF NITROGEN FROM THE TISSUES IN FASTING*

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THE RATE of mobilization of nitrogen from body tissues is, like most physiological processes, influenced profoundly by the concentration of circulating hormones. Of the latter, the thyroid hormone, the adrenal cortical steroids, and the anterior pituitary growth hormone appear to be of particular importance. Participation of the thyroid and the adrenal cortex in protein mobilization has been emphasized by a variety of clinical and laboratory investigations. Generally, the effects of the thyroid and adrenal cortical hormones have been assessed by measurements of the level of nitrogen excretion, and, in clinical and experimental diabetes, the quantity of glucose in the urine. Critical evaluation of the role of growth hormone in protein metabolism has awaited isolation of the growth-promoting principle in purified form.

The source of the urinary nitrogen in circumstances of augmented thyroid or adrenal cortical activity has long interested investigators. This question has been related frequently to the existence in the organism of storage or deposit protein. Solution of these problems has been sought in studies in fasting normal or diabetic animals, since in these circumstances it is possible to eliminate the variable of nitrogen derived from dietary sources. Detailed data of changes in organ size during fasting have been compiled in the classical studies of Jackson and his collaborators (1925). The rate of protein loss from various organs of the rat during fasting was examined by Addis, Poo and Lew (1936a), who found that after seven days of starvation, the liver suffered the greatest relative depletion, losing 40 per cent of its protein. Muscle, skin, and skeleton yielded only 8 per cent each, and brain only 5 per cent, of their protein. However, because of their greater mass, muscle, skin and skeleton together contributed almost two-thirds of the protein that was sacrificed. A fast of two days reduced the liver protein by some 20 per cent, while 4 per cent of the protein of the rest of the body was lost (Addis, Poo and Lew, 1936b).

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It would appear that significant reserves of protein are available for rapid utilization during periods of protein deprivation, and that the rate of contribution of nitrogen by the liver is particularly high.

Since the secretion of the thyroid gland may exert a direct metabolic influence on every cell of the organism, it may be assumed tentatively that this endocrine gland affects the rate of loss of nitrogen from all tissues during starvation. However, a more specific tissue effect is indicated for the hormones of the adrenal cortex. Recent studies (White and Dougherty, 1946; Dougherty and White, 1947) have suggested that these steroid hormones have the lymphocyte as one end-cell of their action. The rate of lymphocyte dissolution, and therefore of the liberation of lymphocyte constituents, including protein, to other body tissues and fluids, is under the control of the adrenal cortex, the secretory level of which is determined by the concentration of circulating pituitary adrenotrophic hormone. The action of the adrenal cortical steroids on lymphocytes may be seen in individual cells, i.e., in the blood or in the gastrointestinal tract, but is most strikingly evident in aggregates of lymphocytes, the lymphoid organs (Dougherty and White, 1945a). Dissolution or involution of lymphoid tissue may occur not only as a result of the administration of pituitary adrenotrophic or adrenal cortical steroid hormones, but also following a wide variety of nonspecific, unrelated physical and chemical stimuli (Selye, 1946) which are known to augment pituitary-adrenal cortical secretion (Sayers, Sayers, Fry, White and Long, 1944).

One of the classical methods of inducing lymphoid tissue involution is that of partial or complete chronic inanition (see Andreasen, 1943, for review of literature). Since restricted food intake activates pituitary-adrenal cortical secretion, the role of this endocrine mechanism in the lymphoid tissue involution of fasting was investigated (Dougherty and White, 1945b). When normal mice are fasted for a period of 48 hours, a marked decrease occurs in the weight and the nitrogen content of the lymphoid tissue. This is the classical lymphoid tissue involution of inanition. At the same time there occurs the often observed loss of weight and nitrogen from the liver and remainder of the body. However, adrenalectomized mice fasted for a comparable period of time, show no loss in lymphoid tissue weight or nitrogen content, although decreases in the liver and other tissues of the body are seen as in the non-operated, fasted mice. Therefore, in the absence of the adrenals, the fasting organism is unable to obtain the direly needed lymphoid tissue nitrogen. Tables I and II contain data illustrating these points. It is interesting to note from Table I that, in agreement with the results of Addis, Poo and Lew (1936b) in the rat, a 48 hour fast in the normal mouse produces approximately a 21 per cent loss of nitrogen from the liver. In contrast to the rat, however, which has a 4 per cent loss of nitrogen from the carcass during a 48 hour fast, the carcass nitrogen loss in the mouse during a similar

fasting period is 12.2 per cent. Of particular interest is the observation that the extent of nitrogen loss from lymphoid tissue during the same period is 31 per cent. Thus lymphoid tissue suffers an even greater relative nitrogen depletion in fasting than does the liver which has generally been considered to have the most significant and readily available stores of nitrogen for periods of protein deprivation (Peters and Van Slyke, 1946). Andreasen (1943) has observed an early, marked atrophy of the lymphoid tissue of the rat during prolonged starvation. Maximum thymus involution occurred three days after initiation of the fasting period.

The effect of prolonging the fasting period to 96 hours has also been studied (Table I). In the normal mouse, extension of the fast to 96 hours produces a further reduction in weight and nitrogen of all tissues studied. The degree of lymphoid tissue nitrogen loss in a normal mouse as a result of a 96 hour fast has been considered maximal since mice often failed to survive longer periods of fasting. Adrenalectomized mice, however, did not lose significant amounts of lymphoid tissue nitrogen during a 96 hour fast (Table II). Selye (1936) observed that adrenalectomy in the rat prevented the marked thymic involution caused by a fast of 96 hours.

Further evidence for the striking role of the adrenal cortex in the lymphoid tissue involution of fasting is seen by the accentuated decline in lymphoid structure weight and nitrogen content which can be produced in the fasting animal by the injection of adrenal cortical steroids. The data in the tables show that mice which have been injected with adrenal cortical steroid hormones during the course of a 48 hour fast have significantly lower lymphoid tissue weight and nitrogen than those seen in mice subjected only to a 48 hour fasting period. Indeed, the lymphoid tissue nitrogen loss of the fasted, hormone treated animals was slightly greater than that considered as maximal in the normal mouse fasted for 96 hours. Furthermore, the apparent specificity of the lymphocyte as an end cell for adrenal cortical steroid hormone action receives additional emphasis by the failure of hormone injection, superimposed on fasting, to alter significantly the values obtained from carcass analysis of the 48 hour fasting mouse (Table II).

Not only is there no decline in the weight and nitrogen content of the lymphoid tissue in the 48 hour fasted, adrenalectomized mouse, but the data suggest that under these conditions an actual increase occurs in the weight and nitrogen content of this tissue. Since this takes place during complete food restriction, the increase must have resulted from the movement of nitrogen from extra-lymphoid structures. This led to an examination of the role of the thyroid gland in the rate of nitrogen loss from tissues during fasting inasmuch as it is well known that hyperthyroidism is accompanied by hypertrophy of lymphoid structures. The role of the thyroid in the mobilization of nitrogen from body tissues may be seen in comparisons of data for

TABLE I. INFLUENCE OF THE ADRENAL CORTEX AND THE THYROID ON THE LOSS OF WEIGHT AND NITROGEN OF TISSUES IN FASTING MICE*

Type of animal	No. of mice	Body weight loss	Liver weight	Lymphoid tissue weight	Liver nitrogen	Lymphoid tissue nitrogen	Carcass nitrogen
		gm.	gm.	gm.	mg.	mg.	gm.
Control, fed	24	—	5.59±0.17	1.18±0.04	179±4.9	30.2±1.3	2.79±0.03
Unoperated, fasted 48 hr.	13	4.5	4.98±0.16	0.74±0.05	140±4.1	20.8±1.0	2.45±0.06
Adrenalectomized, fasted 48 hr.	10	1.5	5.15±0.16	1.38±0.03	148±7.6	36.6±1.0	2.67±0.05
Unoperated, fasted 96 hr.	10	6.7	3.65±0.12	0.57±0.04	129±4.4	16.6±1.2	2.31±0.08
Adrenalectomized, fasted 96 hr.	10	3.1	4.40±0.20	1.12±0.06	138±3.8	29.1±1.3	2.33±0.04
Unoperated, fasted 48 hr., injected with adrenal cortical steroids	10	4.2	5.21±0.18	0.59±0.04	153±4.3	15.5±1.1	2.57±0.06
Thyroidectomized, fasted 48 hr.	10	3.0	4.36±0.29	0.58±0.05	148±9.4	15.8±1.1	2.84±0.06
Thyroidectomized, fasted 48 hr., injected with adrenal cortical steroids	14	2.8	4.58±0.16	0.56±0.03	153±3.9	15.2±0.7	2.73±0.04
Thyroidectomized-adrenalectomized, fasted 48 hr.	11	1.4	5.22±0.13	1.11±0.03	166±5.0	28.5±0.9	2.81±0.05
Adrenalectomized, fasted 48 hr., injected with adrenal cortical steroids	12	3.6	4.66±0.07	0.61±0.02	156±6.7	16.5±0.7	2.56±0.01
Adrenalectomized, fasted 48 hr., injected with thyroxine	9	2.2	5.89±0.19	1.07±0.07	133±2.3	25.7±1.6	2.45±0.02

* All tissue weight and nitrogen values are expressed in terms of 100 gm. of body weight at beginning of experimental period. Values are means and standard errors.

TABLE II. COMPARISONS OF THE ALTERATIONS IN WEIGHT AND NITROGEN OF TISSUES OF FASTING MICE AS INFLUENCED BY ADRENAL CORTICAL AND THYROID ACTIVITY*

Types of animals compared	Liver weight	Lymphoid tissue weight	Liver nitrogen	Lymphoid tissue nitrogen	Carcass nitrogen
	gm.	gm.	mg.	mg.	gm.
Control, fed vs. unoperated, fasted 48 hr.	−0.61 ±0.21	−0.44 ±0.07	−39.0 ± 6.4	− 9.4 ±1.6	+0.34 ±0.06
Control, fed vs. adrenalectomized, fasted 48 hr.	−0.44 ±0.21	+0.20 ±0.05	−31.0 ± 9.0	+ 6.4 ±1.6	−0.12 ±0.06
Control, fed vs. adrenalectomized, fasted 96 hr.	−1.19 ±0.26	−0.06 ±0.07	−41.0 ± 6.2	− 1.1 ±1.5	−0.46 ±0.05
Unoperated, fasted 48 hr. vs. unoperated, fasted 48 hr., injected with adrenal cortical steroids	+0.23 ±0.24	−0.15 ±0.06	+13.0 ± 5.9	− 5.3 ±1.5	+0.12 ±0.08
Control, fed vs. thyroidectomized, fasted 48 hr.	−1.23 ±0.34	−0.60 ±0.06	−31.0 ±10.5	−14.4 ±1.7	+0.05 ±0.06
Unoperated, fasted 48 hr. vs. thyroidectomized, fasted 48 hr.	−0.62 ±0.31	−0.16 ±0.07	+ 8.0 ±10.1	− 5.0 ±1.5	+0.39 ±0.08
Control, fed vs. thyroidectomized, fasted 48 hr., injected with adrenal cortical steroids	−1.01 ±0.23	−0.62 ±0.05	−26.0 ± 6.2	−15.0 ±1.8	−0.06 ±0.05
Thyroidectomized, fasted 48 hr. vs. thyroidectomized, fasted 48 hr., injected with adrenal cortical steroids	+0.22 ±0.39	−0.02 ±0.06	+ 5.0 ±10.6	− 0.6 ±1.3	−0.12 ±0.07
Control, fed vs. thyroidectomized-adrenalectomized, fasted 48 hr.	−0.37 ±0.21	−0.07 ±0.05	−13.0 ± 7.0	− 1.7 ±1.6	+0.02 ±0.06
Adrenalectomized, fasted 48 hr. vs. adrenalectomized, fasted 48 hr., injected with adrenal cortical steroids	−0.49 ±0.18	−0.77 ±0.04	+12.0 ±10.8	−20.1 ±1.2	−0.11 ±0.05
Adrenalectomized, fasted 48 hr., injected with adrenal cortical steroids, vs. adrenalectomized, fasted 48 hr., injected with thyroxine	+1.23 ±0.20	+0.46 ±0.07	−23.0 ± 7.0	+ 9.2 ±1.8	−0.11 ±0.02

* The values in this table are arithmetical differences of the means, and standard errors of the differences, obtained by comparisons of the data in Table I for the indicated groups of animals. The standard errors of the differences were calculated from the standard errors of the means in Table I, using the expression, S.E. of differences $= \sqrt{(S.E._1)^2 + (S.E._2)^2}$.

The differences of the means were considered significant when they exceeded by at least three-fold the standard errors of the differences.

fed, control mice with those for thyroidectomized animals fasted for 48 hours (Table II). It is apparent that in the absence of thyroid secretion, the fasting mouse suffers no loss of nitrogen from the major portion of his tissues which are grouped together under the term, carcass. This reemphasizes the important role which the thyroid plays in the mobilization of nitrogen stores of the carcass during fasting. The data also support the suggestion that the lymphoid tissue growth seen in 48 hour fasted, adrenalectomized mice may have resulted from a transfer of nitrogen from the carcass to lymphoid tissue, and that the rate of this process is under thyroid control. Further evidence for this conclusion is also derived from a comparison of lymphoid tissue weight and nitrogen values between groups of unoperated and of thyroidectomized mice, all subjected to a 48 hour fast. In the absence of the thyroid, the involution of lymphoid tissue, controlled by adrenal cortical secretion, is proceeding uncompensated by a thyroid-regulated replenishment by nitrogen from carcass, and, as a result, lymphoid tissue involution is manifested even more strikingly. The loss of lymphoid tissue nitrogen in these circumstances is as great as that seen in normal mice showing maximal loss as a result of a 96 hour fast, or in normal animals fasted 48 hours and injected with adrenal cortical steroids. In the latter case, it would appear that the animal's thyroid is unable to compensate adequately for the increased level of circulating adrenal cortical steroids, and, as a consequence, lymphoid tissue replenishment is insufficient and the nitrogen content of this tissue is reduced maximally.

Added evidence for the role of the adrenal cortex in the specific mobilization of lymphoid tissue nitrogen, and that of the thyroid in the regulation of the rate of nitrogen removal from carcass stores, is obtained from comparisons of data for control, fed animals with those for thyroidectomized mice fasted 48 hours and injected with adrenal cortical steroids. Despite the catabolic effects of these steroid hormones in protein metabolism, their administration to fasting, thyroidectomized mice does not produce a significant decrease in carcass nitrogen. Furthermore, comparison of the data for groups of thyroidectomized, fasted mice with those for thyroidectomized, fasted mice injected with adrenal cortical steroids, reveals that in the absence of the thyroid, the animals' own adrenals are operating at a maximal rate and the supplying of external sources of adrenal cortical hormones produces no further involution of lymphoid tissues. Finally, the failure of the adrenalectomized, thyroidectomized mice to show a significant loss in carcass nitrogen and in lymphoid tissue weight and nitrogen when subjected to a 48 hour fast is further evidence for the role of these two endocrine glands in the regulation of the rate of nitrogen loss from tissues during fasting. The lymphoid cells appear to be an end organ of adrenal cortical steriod action, whereas the mass of muscle tissue is affected chiefly by the secretion of the thyroid gland. On the other hand, the data in the tables indicate that the rate of

nitrogen loss from liver tissue during fasting is affected by both adrenal cortical and thyroid secretions. Liver nitrogen decreases to the same extent in fasting adrenalectomized, or thyroidectomized animals, but in the absence of both of these endocrine glands the slight loss in liver nitrogen produced by fasting is not statistically significant.

Further evidence for an interrelationship between the secretions of the adrenal cortex and the thyroid is seen from a comparison of the data for adrenalectomized mice fasted 48 hours and injected with adrenal cortical steroids with values for adrenalectomized animals subjected only to the fasting period. The responsiveness of the thyroid to augmented adrenal cortical activity is observed in the demonstration that the loss of carcass nitrogen is greater in adrenalectomized, fasting mice receiving the steroid hormones than in the operated, fasting animals not injected with extract. It may also be seen from Table II that the level of carcass nitrogen reached after a 48 hour fast in adrenalectomized mice given adrenal cortical steroids is not as low as that found after a 48 hour fast in operated animals given thyroxine. Also, the nitrogen content of the carcass of normal mice fasted for 48 hours is distinctly lower than that of adrenalectomized mice fasted for a similar period of time, but the lower value can be attained in the fasted, adrenalectomized animal if thyroxine is administered (carcass values for unoperated mice fasted 48 hours are the same as those for adrenalectomized mice fasted 48 hours and given thyroxine, Table I). The data all support the suggestion that adrenal cortical secretion has an augmenting influence on thyroid activity. However, the reverse relationship, i.e., thyroid secretion as an accelerator of adrenal cortical activity, does not appear to obtain inasmuch as lymphoid tissue weight and nitrogen reach as low levels in the thyroidectomized animal fasted for 48 hours as in similarly operated mice receiving adrenal cortical steroids during the fasting period. The maximum involution of lymphoid tissue seen as a result of adrenal cortical stimulation is thus not dependent on thyroid secretion. It should be pointed out, however, that in the normal, fed animal thyroid hormone administration may produce an acute dissolution of lymphocytes (Latta and Brenner, 1934). In this respect, the thyroid effect may be a non-specific stimulator of pituitary-adrenal cortical secretion. This may be of significance in the normal, fed organism.

The data which have been obtained reemphasize the rapid mobilization of body nitrogen which occurs in the fasting organism. This mobilization takes place at the expense of all the tissues of the body, with the possible exception of the eyes, testicles and adrenals (Addis, Poo and Lew, 1936a). The rate of nitrogen loss from the tissues during fasting is influenced markedly by the level of secretion of the endocrine glands, notably the thyroid and the adrenal cortex which, in turn, are dependent for their regulation on the trophic secretions of the anterior pituitary gland. The relatively large contribution which the

muscle, as represented by carcass analysis, makes to nitrogen mobilization is indicated to be under thyroid control, while the steroid hormones of the adrenal cortex appear to mobilize specifically the nitrogen of the lymphoid tissue. The latter tissue is characterized by a rapid nitrogen loss during fasting, exceeding in rate and degree that seen in the liver. Nitrogen loss from the liver proceeds at a rapid rate, appears to continue in the absence of either adrenals or thyroid, but is greatly retarded in rate in the absence of both of these endocrine glands.

The suggestion that nitrogen mobilization from the carcass is under thyroid control has been made as a result of the inability of adrenal cortical steroids to lower the carcass nitrogen of the thyroidectomized, fasted mouse. The data obtained do not permit the distinction between whether the thyroid hormone is specifically concerned with carcass nitrogen mobilization, or whether the presence of small amounts of thyroid hormone is required for the manifestation of carcass nitrogen catabolism by other hormones, including those of the adrenal cortex. An answer to this question will be sought in future investigations. Ingle and his associates have emphasized the possibility of this type of dual interpretation as a result of experiments demonstrating that the presence of adrenal cortical hormones is required in adrenalectomized animals for a manifestation of the diabetogenic activity of stilbestrol (Ingle, 1943) and the negative nitrogen balance following fractures (Ingle, Ward and Kuizenga, 1947), although neither response is caused specifically by increased adrenal cortical secretion.

It would appear that a degree of balance obtains between the thyroid and the adrenal cortex in their effects on nitrogen loss during fasting. Restricted or complete inanition is a potent activator of pituitary-adrenal cortical secretion. As a result of an increase in the concentration of circulating adrenal cortical steroid hormones, thyroid secretion is accelerated. A portion of the nitrogen released from muscle stores due to augmentation of thyroid activity may be utilized to restore depleted lymphoid nitrogen. Thus it is implied that there is a redistribution of nitrogen stores, i.e., a translocation of nitrogen from muscle to lymphoid tissue. This was suggested from the fact that the lymphoid tissue nitrogen is significantly higher in the adrenalectomized mouse fasted 48 hours than in the fed, unoperated animal. Further indication of an interchange of nitrogen among the tissues of the fasting animal is seen in mice which were injected with adrenal cortical steroids. Hormone administration to either normal, adrenalectomized, or thyroidectomized mice fasted 48 hours produced an increase in liver nitrogen, as compared to similar groups of fasted mice not receiving adrenal cortical extract. However, the magnitude of the alterations in liver nitrogen was not statistically significant, and the evaluation of this trend must await determinations of the rates of nitrogen turnover under these experimental conditions. The simple

device of employing the fasting animal permits an initial evaluation of the rate of loss of nitrogen from certain structures, the contribution of this nitrogen to the maintenance of other tissue nitrogen stores, and the regulation of these processes by various endocrine glands. It is suggested that nitrogen lost from tissues during fasting may be utilized not only for catabolic purposes but also for maintaining the integrity of other tissues which may be vital for the body economy.

Although lymphoid tissue suffers the greatest relative nitrogen loss (31 per cent) during a 48 hour fast, followed in degree by liver (21 per cent) and carcass (12 per cent), it is evident that the carcass makes the greatest absolute contribution of nitrogen since this tissue represents more than 90 per cent of the total weight of the experimental animals. Therefore, the rate of contribution of lymphoid tissue to the nitrogen loss of injury and other types of stress may be exceedingly high, but the total amount of nitrogen contributed is of less relative significance. What proportion of the total catabolic nitrogen may be released to the metabolic pool via the lymphoid structures, i.e., as a result of other sources of tissue nitrogen being first translocated to lymphoid organs before release for catabolic purposes, remains to be studied in future investigations.

The function of the adrenal cortex in the mobilization of tissue nitrogen has been demonstrated by numerous other investigators, particularly in clinical and experimental diabetes. Evans (1936) observed that the excretion of nitrogen in phlorhizinized rats was greatly reduced following adrenalectomy. In the same year, Long and Lukens (1936) made the striking observation that adrenalectomy in the depancreatized cat was followed by an amelioration of the diabetes and a decline in the high urinary nitrogen seen in the diabetic organism. Since these classical studies, a variety of investigations have confirmed the evidence for a role of the adrenal cortex in protein catabolism. The nature of this role appeared to be one of controlling the rate of mobilization of endogenous protein, since the hypophysectomized (Soskin, Mirsky, Zimmerman and Crohn, 1935) or adrenalectomized animal (Wells and Kendall, 1940) can utilize exogenous protein. However, despite this evidence, it was concluded that the influence of the anterior pituitary gland on gluconeogenesis was to facilitate the conversion of tissue proteins to the amino acid stage (Soskin, Mirsky, Zimmerman and Crohn, 1935), and that the adrenal cortical steroids exerted a specific antagonism to insulin (Wells and Kendall, 1940). In view of the data obtained in the normal and adrenalectomized fasted mice, and the demonstrated influence of adrenal cortical secretion on thyroid activity, it would appear that adrenal cortical hormones exert a direct influence on the mobilization of lymphoid nitrogen and an indirect action, via the thyroid, on the mobilization of carcass nitrogen. This indirect effect of adrenal cortical secretion on the level of thyroid activity may be of significance in relation to the need for adrenal cortical hormones for the manifestation of the diabetogenic activity of stil-

bestrol (Ingle, 1943) and the negative nitrogen balance following fractures (Ingle, Ward and Kuizenga, 1947) or trauma (Toby and Noble, 1947). Although these responses are not caused specifically by increased adrenal cortical secretion, the presence of these steroid hormones may be necessary to permit manifestation of maximum thyroid control of nitrogen mobilization from the organism's largest nitrogen stores, the carcass.

The administration of thyroid or thyroxine in a wide variety of experimental conditions has been demonstrated to produce an increased nitrogen excretion. It is clear that in the fasting organism this nitrogen has its origin in tissue protein and other tissue nitrogenous constituents which are mobilized and catabolized at an increased rate as a result of hormone administration. This has been seen in both clinical and experimental diabetes. The literature concerning the role of the thyroid in augmenting glucose and nitrogen excretion in diabetes has been reviewed recently by Houssay (1946). Of particular interest to the present discussion is the protection against experimental diabetes afforded by thyroidectomy. Houssay (1946) proposes several possible explanations for the protective action of thyroidectomy, but does not include one which seems indicated from the data obtained with thyroidectomized, fasted mice, viz., a decreased rate of mobilization of the large tissue reserves of protein. This would result in a diminished supply of new carbohydrate precursors.

The complementary action of adrenal cortical and thyroid secretions in the mobilization of tissue nitrogen was suggested by Wells and Chapman (1940) from studies of hypophysectomized, phlorhizinized rats. While adrenal replacement therapy, in the form of potent adrenal cortical steroids, restored the rate of glucose and nitrogen excretion almost to the level seen in the unoperated, phlorhizinized animal, a further slight augmentation in the excretion of both of these urinary constituents was reported when the rats were also given thyrotrophic hormone. Although the data obtained by Wells and Chapman (1940) are not statistically significant for the small groups of animals employed, the suggestion by those authors of a synergistic action of the adrenal cortex and thyroid in phlorhizin diabetes is supported by the present investigation. Sternheimer (1939) reported that during the 48 hour period following a single injection of thyroxine in rats, there was a continuing increase in liver protein, probably as a result of mobilization of protein from peripheral tissues.

The conclusion that the thyroid and the adrenal cortex control the rate of nitrogen loss from tissues does not imply that this process ceases completely in the absence of these endocrine glands. It is striking that the thyroidectomized-adrenalectomized mice fasted for 48 hours showed no significant loss of nitrogen from the three types of tissues studied. If nitrogen turnover of tissues is continuing in these animals, then it would appear that there must be some balanced exchange among the structures of the organism. It is unlikely that ni-

trogen turnover has ceased entirely in the tissues of adrenalectomized-thyroidectomized mice. Further analysis of this question is being sought with isotope-labelled proteins.

It is apparent that each of the categories of tissues which has been studied makes a significant contribution to the total nitrogen which is mobilized during fasting, and probably during other circumstances of stress. Thus, in all of the diverse structures of the organism may reside a portion of the deposit or reserve nitrogen which long has interested investigators. In relation to this nitrogen, tissues differ chiefly from one another in the rate at which each makes its contribution of nitrogen to the metabolic pool, and this rate is influenced by hormonal secretions. It would seem unnecessary, therefore, to characterize deposit or reserve nitrogen as distinct from tissue or structural nitrogen. In response to a suitable stimulus, cells of diverse types may yield cellular consitutents, including nitrogen-containing substances, to the extra-cellular environment and thus to subsequent metabolic transformations. With this viewpoint, storage portein becomes identical with structural protein, and the attempt to distinguish between these is largely an effort to describe the relative degrees to which factors influencing protein catabolism, e.g., hormones, may affect different histological structures.

EXPERIMENTAL

Mice of both sexes and of the inbred CBA strain (Strong) were used. All animals weighed between 18 and 22 gm. at the beginning of an experimental period, and were from 8 to 11 weeks of age. The fasting animals were kept in individual metabolism cages constructed to prevent access to excreta. Drinking water was available during the fasting periods.

Operative procedures, i.e., adrenalectomy and thyroidectomy, were performed 24 hours before the beginning of an experimental period. Ether anesthesia was used for adrenalectomy, and amytal both for thyroidectomy and for the double operation, i.e., adrenalectomy and thyroidectomy. Each animal subjected to adrenalectomy, or to adrenalectomy and thyroidectomy, received immediately postoperatively a single, subcutaneous injection of 0.25 mg. of desoxycorticosterone acetate in sesame oil (Schering). The thyroidectomized animals were untreated prior to the start of the fasting period. All mice, operated and unoperated, had free access to food (Laboratory Chow) and water until the start of the fasting period. The operated animals may have had a diminished food intake in the 24 hour postoperative period so that the actual experimental fasting period may represent a more severe food restriction than intended.

Adrenal cortical steroids in oil (Upjohn) and L-thyroxine (Squibb) were used as hormone preparations. The thyroxine was employed in aqueous solution (2.5 mg/cc.). Mice receiving adrenal cortical steroids during a 48 hour fast were injected at 7 and at 24 hours after initiation

of the fast, 0.25 ml. of adrenal cortical steroids in oil being given subcutaneously at each injection. Mice receiving thyroxine were injected subcutaneously at similar time intervals during the 48 hour fast, and received 0.5 mg. thyroxine at each injection.

At the end of the experimental period, the mice were anesthetized with nembutal preparatory to tissue dissection. As representative lymphoid tissue, the following tissues were pooled for weighing and analysis: paired inguinal and axillary lymph nodes, mesenteric node, six cervical nodes (three from each side of the neck), thymus and spleen. The liver was then removed for weighing and analysis, and the remainder of the animal was considered carcass and included the skin and hair. The total lymphoid tissue was taken for Kjeldahl digestion, with suitable aliquots then being employed in triplicate for completion of the analysis. In the case of the liver, triplicate small portions of tissue were excised from several areas of the organ, weighed and digested for individual nitrogen analyses. The portion of the animal which was termed carcass was finely minced by use of a suitable scissors until a uniform appearing tissue mince was obtained. The latter was then mixed thoroughly, and triplicate samples, taken from different areas of the minced tissue, were each weighed and digested.

Semi-micro Kjeldahl analysis for total nitrogen was employed. Two ml. of concentrated sulfuric acid were used for digestion, with a selenium granule as catalyst, and several drops of superoxol were employed to complete the digestion. The analytical value for each category of tissue for each mouse represents the average of triplicate analyses.

SUMMARY

The influence of the adrenal cortex and the thyroid in regulating the rate of mobilization of tissue nitrogen has been studied in fasting normal, adrenalectomized, thyroidectomized, and adrenalectomized-thyroidectomized, mice. Studies have also been conducted in fasting, normal and adrenalectomized mice injected with adrenal cortical steroids, in fasting, adrenalectomized mice injected with thyroxine, and in fasting, thyroidectomized mice injected with adrenal cortical steroids. Weight and nitrogen analyses of representative lymphoid organs, liver and of the remainder of the body, which has been termed carcass, have permitted an evaluation of the adrenal cortical and thyroid regulation of the nitrogen contribution which each of these types of tissues makes during a period of fasting.

In the normal mouse subjected to a 48 hour fast, there is a loss of 31 per cent of the nitrogen of lymphoid tissue, 21 per cent of the liver nitrogen and 12 per cent of the carcass nitrogen. From the standpoint of relative losses, therefore, lymphoid tissue makes an even more significant contribution to nitrogen mobilization than does the liver. However, the total quantity of nitrogen contributed by carcass exceeds that of the lymphoid tissue and the liver.

The steroid hormones of the adrenal cortex, whose secretory rate is under control of pituitary adrenotrophin, regulate specifically the rate of release of nitrogen from lymphoid tissue. The fasting, adrenalectomized animal draws significantly upon liver and carcass nitrogen only. Thyroid secretion controls the rate of nitrogen loss from carcass; the fasting, thyroidectomized mouse obtains significant quantities of nitrogen only from liver and lymphoid tissues. The adrenalectomized-thyroidectomized fasting mouse derives a small quantity of nitrogen from liver tissue only.

Data have been obtained to support the suggestion that adrenal cortical secretion is one of the factors regulating thyroid activity. On the other hand, adrenal cortical activity in these experiments was not dependent on the thyroid, since maximum involution of lymphoid tissue was evident in fasting, thyroidectomized animals.

The significance and relationships of the role of the adrenal cortical and thyroid hormones in the mobilization of nitrogen have been considered in the fasting mouse. Additional or varying interpretations and integrations may obtain in other species, and in well-fed animals.

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A CYTOCHEMICAL STUDY OF THE ADRENAL CORTEX IN HYPO- AND HYPERTHYROIDISM[1]

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THE ADRENAL cortex enlarges in experimentally hyperthyroid animals and shrinks in animals made hypothyroid by thyroidectomy or by feeding antithyroid drugs such as thiouracil or thiourea (Marine, 1935; Leblond and Hoff, 1944; Baumann and Marine, 1945). It has not been ascertained whether these changes in the adrenal gland are caused by an altered reactivity of the cortex or whether they reflect modified rates of production of pituitary adrenotropin. Baumann and Marine state that the adrenal cortex "exercises an inhibitory control over the thyroid gland" and interpret the involution of the cortex after administration of thiouracil as an "attempt to compensate for loss of the thyroid secretion." There has been little support for their belief that the connection between these two glands is so direct. To us it seems more reasonable to interject the pituitary gland and its specific hormones into any suspected thyroid-adrenal inter-relationship.

Recent cytochemical studies (Deane and Greep, 1946; Deane and Shaw, 1947; Greep and Deane, 1947) of the adrenal cortex of animals with altered cortical function indicate that the adrenal cortex contains two secreting zones which serve different purposes. Our evidence supports the following hypothesis: The zona glomerulosa secretes 11-desoxycorticosteroids which regulate sodium loss, whereas the zona fasciculata secretes 11-oxycorticosteroids which affect the metabolism of organic substances. Only the fasciculata appears to be controlled by the pituitary gland. In the light of these relationships, it was of interest to determine whether the adrenal changes induced by altered thyroid activity involve the glomerulosa or the fasciculata, or both.

MATERIALS AND METHODS

Young adult male rats of the Long-Evans and Sprague-Dawley strains were used. The diet consisted of Purina fox chow. All animals were allowed to eat and drink *ad libitum*.

Hypothyroidism was produced by two means. Eleven rats, in one group,

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were thyro-parathyroidectomized under nembutal anesthesia, while in 51 rats, in a second group, thyroid function was inhibited by the administration of 2-thiouracil (American Cyanamid). Forty-three rats of the latter group were given a 0.1 per cent solution of drug in the drinking water, receiving approximately 25 mg. daily. The remaining eight animals were fed a diet of ground chow containing 0.5 per cent thiouracil so that their daily drug intake was roughly 75 mg. Animals in these groups were killed at 7, 14, 28 and 56 days, respectively, after the operation or after beginning thiouracil treatment. Eleven of the rats, maintained on thiouracil in the drinking water, were killed at the end of 15 weeks.[2]

For determining the cytochemical changes in the adrenal cortex of hyperthyroid animals, a group of 8 rats were fed thyroid powder (Armour) mixed with ground chow in the concentration of 0.5 per cent, and 8 received twice that concentration. Three rats receiving 1 per cent thyroid powder succumbed in the 4th week, and of those receiving 0.5 per cent thyroid powder one died in the 6th week. Autopsies were performed at 7, 14, 28 and 56 days.

Control rats of the same age and strain were killed with the several groups.

At autopsy, the paired adrenal glands, the thymus gland, and, if present, the thyroid glands were weighed on a Roller-Smith precision balance. One adrenal was fixed in 10 per cent neutralized formalin for at least 48 hours, then sectioned on the freezing microtome at 15 μ. One section was stained with sudan IV and Harris' hematoxylin, another with sudan black B, and a third by the Schiff plasmal method; these were mounted in glycerin-jelly. Two sections were mounted unstained in glycerin, one of which had been extracted for one-half hour in cold acetone. The latter were viewed under the polarizing and fluorescence microscopes for acetone-soluble birefringent and autofluorescent materials. Ketosteroids are characterized by being sudanophilic, Schiff-positive, autofluorescent, birefringent and acetone-soluble; no other single substance is known to possess all of these characteristics. Consequently it is probable that any lipid droplet displaying the whole battery of reactions contains ketosteroids (Dempsey and Wislocki, 1946).

RESULTS

Control Rats

The normal weight of the paired adrenal glands in the young adult male rats was 15–19 mg. per 100 grams body weight (Tables 1, 2 and 3). The thymus weighed somewhat over 200 mg. per cent and the thyroid about 8.5 mg. per cent.

We have described and illustrated the histology and cytochemistry of the normal rat's adrenal cortex in some detail elsewhere (Deane and Greep, 1946; Greep and Deane, 1947). To recapitulate briefly, the cortex possesses a narrow zona glomerulosa containing a large amount of ketosteroid (i.e., sudanophilic, Schiff-positive, birefringent and autofluorescent lipid), a lipid-free transitional zone, a broad zona fascicu-

[2] We are indebted to Dr. William L. Money of the Biological Laboratories, Harvard University, for these rats.

lata which contains a large amount of ketosteroid in its outer portion and less centrally, and, finally, an ill-defined zona reticularis containing scattered fatty cells, most of which are negative for ketosteroids (Figs. 2 and 3).

Hypothyroidism

After surgical thyroidectomy the adrenal glands shrink from the normal 19 to 13 mg. per cent and the thymus gland from 160 to 70 mg. per cent (Table 1).

TABLE 1. EFFECT OF HYPOTHYROIDISM, PRODUCED BY COMPLETE THYROIDECTOMY, ON ADRENAL AND THYMUS WEIGHTS IN ADULT MALE RATS

Operated	No. rats	Weight, grams	Adrenal wt.		Thymus wt.	
			mg.	mg./100 g.	mg.	mg./100 g.
Intact	3	161	30.5	18.9	261	162
7 days	3	145	25.5	17.6	236	163
14 days	3	153	29.1	19.0	236	154
28 days	3	163	27.9	17.1	229	141
56 days	2	159	20.9	13.1	116	73

Histologically the adrenal atrophy is a result of a shrinkage of the cortex, particularly of the zona fasciculata (cf. Figs. 2 and 4). The glomerulosa appears broader than normal and to contain more lipid. The ketosteroid reactions are similarly altered. During the 2 months' experimental period, the lipoidal zone in the fasciculata narrows progressively and suffers a reduction in all ketosteroid reactions. The glomerulosa, on the other hand, gradually becomes abnormally crowded with steroidal material, as revealed particularly in the birefringence preparations (Fig. 5).

After treatment with thiouracil the adrenal and thymus glands atrophy (Table 2), beginning at an earlier time than after thyroidectomy. However, in the group of rats killed after prolonged treatment (15 weeks), some of the adrenals are of normal size; this phenomenon was also noted by Baumann and Marine (1945). The involution of the adrenal and thymus glands is similar whether the dose of thiouracil is 25 or 75 mg. daily, although the latter is more goitrogenic (Table 2).

Histologically the adrenal cortices are atrophied (Figs. 6, 8, 12), except for the glands of some animals treated for 15 weeks, as noted above (Fig. 10). This atrophy is accompanied by an hypertrophy of the medulla, which therefore serves to mask somewhat the decrease in adrenal weight (Marine and Baumann, 1945).

In the cortices of rats receiving about 25 mg. thiouracil daily in the drinking water, the following sequence of changes occurs. At 7 days there is no conspicuous change in the distribution of sudanophil lipid,

TABLE 2. EFFECT OF HYPOTHYROIDISM, DUE TO 2-THIOURACIL, ON ADRENAL, THYMUS AND THYROID WEIGHTS IN ADULT MALE RATS

Treatment	No. rats	Weight, grams	Adrenal wt.		Thymus wt.		Thyroid wt.	
			mg.	mg./100 g.	mg.	mg./100 g.	mg.	mg./100 g.
None	5	158	29.1	18.4	393 (2)	199	13.0	8.2
a. 0.1 per cent thiouracil in H_2O (ca. 25 mg. daily)								
7 days	16	128	21.8	17.8	400 (2)	234	21.5	16.8
14 days	7	173	22.5	13.0	255	147	42.5	24.6
28 days	7	188	21.6	11.5	249	132	51.0	27.1
56 days	2	242	30.0	12.4	301	124	59.9	24.7
15 weeks	11	288	34.6	12.0	202	70	84.3	29.2
b. 0.5 per cent thiouracil in food (ca. 75 mg. daily)								
7 days	2	168	27.2	16.2	337	200	32.4	19.3
14 days	2	168	22.1	13.1	289	172	52.6	31.3
28 days	2	179	23.3	13.0	216	121	70.2	39.2
56 days	2	208	27.5	13.2	284	137	134.0	64.5

although some reduction in its quantity is common in both the glomerulosa and fasciculata. The Schiff reaction and autofluorescence are somewhat subnormal, and birefringence is distinctly reduced, especially in the glomerulosa. By 14 days the zone of sudanophil material in the glomerulosa is clearly narrowed, and the other ketosteroid reac-

Explanation of figures on Plate 1

All photomicrographs are of adrenal glands which were fixed in 10 per cent neutralized formalin for over 48 hours, washed thoroughly, and sectioned at 15 μ on the freezing microtome. ×90.

FIG. 2. Adrenal cortex of a control rat. Sudan IV and hematoxylin. Sudanophilic lipids are particularly rich in the zona glomerulosa and in the outer part of the zona fasciculata, with a lipid-poor transitional zone between. The droplets stain more darkly in the glomerulosa.

FIG. 3. Adrenal cortex of a control rat. Birefringence pattern. There are some birefringent particles in the zona glomerulosa and many in the zona fasciculata. They are mostly coarse, with some fine, dull particles intermixed. The coarse particles are probably a storage steroid, whereas the fine ones indicate secretion (Weaver and Nelson, 1943).

FIG. 4. Adrenal cortex of a rat thyroidectomized for 56 days. Sudan IV and hematoxylin. The fasciculata is conspicuously narrower than in the controls. While the zona glomerulosa contains even more lipid than in the controls, fat is restricted almost entirely to the outer part of the fasciculata, and stains a deeper red than in the controls.

FIG. 5. Adrenal cortex of a rat thyroidectomized for 56 days. Birefringence pattern. The zona glomerulosa is conspicuously crowded with fine birefringent particles, while the zona fasciculata contains little birefringent material, which is mostly coarse in character.

FIG. 6. Adrenal cortex of a rat receiving 0.1 per cent thiouracil in water for 28 days. Sudan IV and hematoxylin. The zona fasciculata is narrower than normal. The glomerulosa is completely devoid of lipid, and that in the fasciculata is relatively sparse.

FIG. 7. Adrenal cortex of a rat receiving 0.1 per cent thiouracil in water for 14 days. Birefringence pattern. Birefringent particles are scarce in the zona glomerulosa and reduced in number in the fasciculata, where they are predominantly coarse.

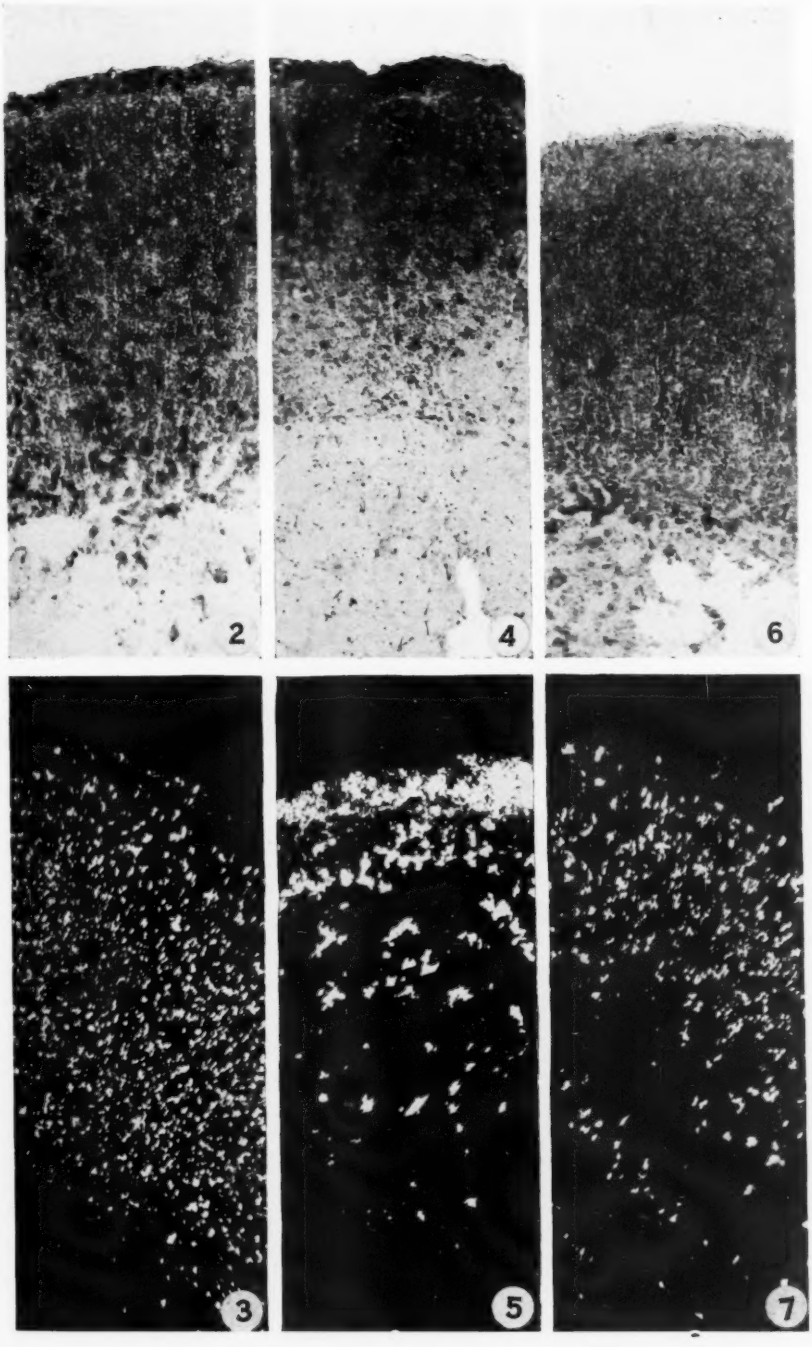


PLATE 1

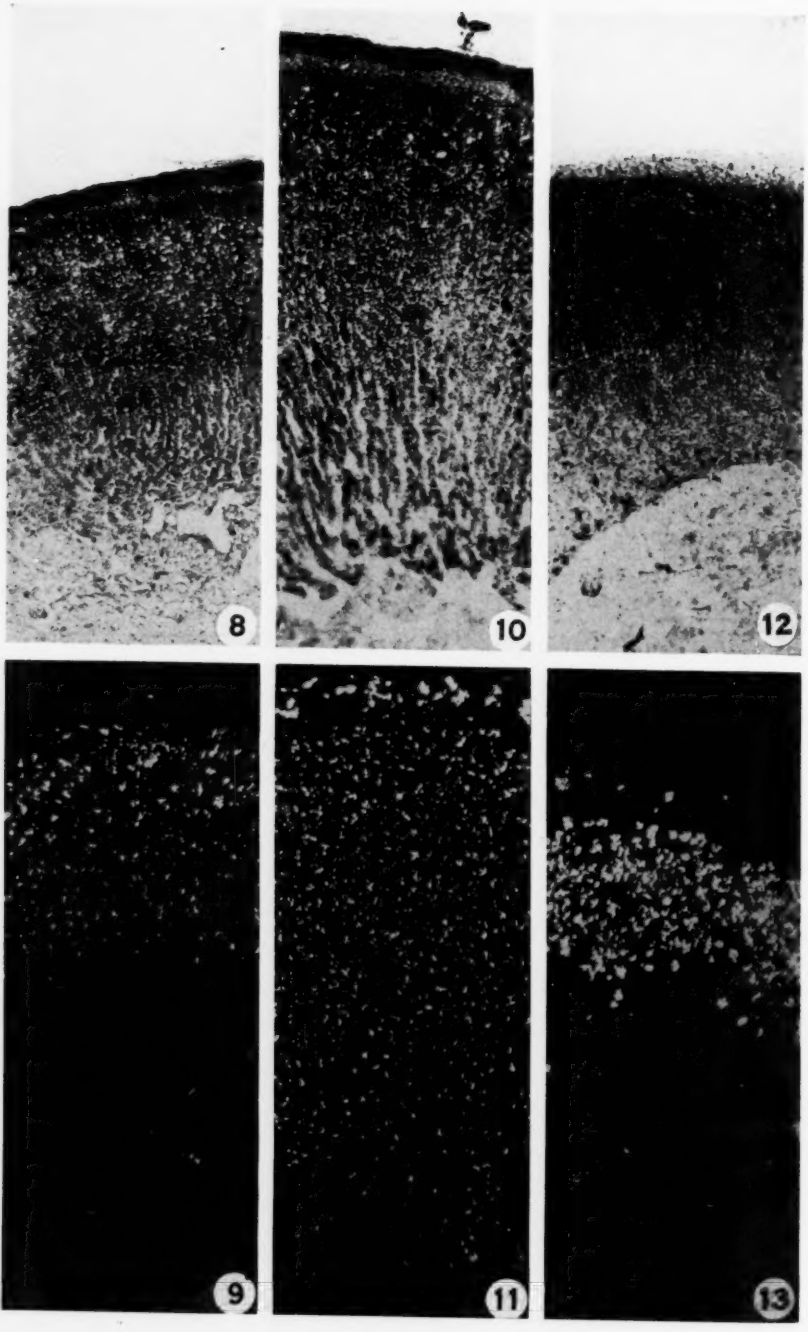


PLATE 2

tions are virtually blank. The amount of lipid and ketosteroid material (Fig. 7) in the fasciculata appears subnormal; there is a shift in the color of the sudan IV staining from the usual orange in this zone to a bright red. Some conspicuously fatty cells, resembling adipose-tissue cells, may be seen, especially in the reticularis (Fig. 7).

At 28 days the zona glomerulosa has generally, but not invariably (Fig. 6), recovered its sudanophilia, in which case the other steroid reactions are of normal intensity. The zona fasciculata, however, contains lipid droplets only in its outer section. The Schiff reaction and autofluorescence are subnormal in this zone, and the birefringent particles, although present in considerable quantity, are almost entirely coarse in appearance. After 56 days of treatment with this dose of thiouracil, the zona glomerulosa appears broad and is filled with intensely sudanophilic droplets, the ketosteroid reactions of which are of normal intensity. The shrunken fasciculata possesses considerable sudanophil lipid but its distribution is somewhat irregular, and the the ketosteroid reactions are essentially similar to those seen after 1 month of treatment.

After 15 weeks' treatment variable conditions exist. In every instance the glomerulosa is intensely sudanophilic and presents normal ketosteroid reactions. In the rats with shrunken adrenals the fasciculata contains a somewhat less than normal amount of lipid (Fig. 9). That which is present stains darkly with sudan IV (Fig. 8), is not particularly reactive with the Schiff reagent or more than moderately autofluorescent, and the birefringent particles are rather widely separated (Fig. 9). In the rats with adrenals of normal size the fasciculata is unusually rich in lipids (Fig. 10), which give strong steroid reactions. The birefringence preparations show a considerable proportion of fine particles (Fig. 11).

Explanation of figures on Plate 2

FIG. 8. Adrenal cortex of a rat receiving 0.1 per cent thiouracil in water for 15 weeks. Sudan IV and hematoxylin. The cortex is considerably shrunken. The zona glomerulosa is crowded with deeply sudanophilic droplets; the fasciculata contains a subnormal amount of fat although this stains unusually deeply.

FIG. 9. Adrenal cortex of a rat receiving 0.1 per cent thiouracil in water for 15 weeks. Birefringence pattern. Both the glomerulosa and outer fasciculata contain birefringent particles, predominantly coarse.

FIG. 10. Adrenal cortex of a second rat receiving 0.1 per cent thiouracil in water for 15 weeks. Sudan IV and hematoxylin. The cortex is essentially normal, both in width and in the distribution of lipids.

FIG. 11. Adrenal cortex of the same rat receiving 0.1 per cent thiouracil in water for 15 weeks. Birefringence pattern. There is a normal quantity of steroid present, with the particles in the glomerulosa mostly coarse but many in the fasciculata fine.

FIG. 12. Adrenal cortex of a rat receiving 0.5 per cent thiouracil in food for 56 days. Sudan IV and hematoxylin. The cortex is conspicuously atrophied. Sudanophilic material is absent from the zona glomerulosa, while that in the outer part of the fasciculata stains a deep red.

FIG. 13. Adrenal cortex of a rat receiving 0.5 per cent thiouracil in food for 56 days. Birefringence pattern. Almost no birefringent particles occur in the zona glomerulosa, and those in the fasciculata are mostly coarse.

The cortices of rats receiving about 75 mg. thiouracil daily in the food present a slightly different sequence of changes from those receiving 25 mg. At 7 days the glomerulosa contains less ketosteroid material than normal; the narrowed fasciculata also contains little lipid, and the lipid-free zone between the glomerulosa and fasciculata is exceptionally broad. At 14 days, however, the lipid in the glomerulosa is somewhat restored, while the fasciculata contains relatively sparse, but markedly sudanophilic, droplets, which are almost negative by the Schiff test and are only slightly autofluorescent. The birefringent particles in this zone are fine. After 28 and 56 days of treatment, the notable features are that the glomerulosa reveals a low lipid content and the outer fasciculata has become filled with dark red sudanophilic droplets (Fig. 12), which are deeply Schiff positive, autofluorescent and coarsely birefringent (Fig. 13).

Hyperthyroidism

Thyroid powder added to the diet at a concentration of 1 per cent causes death in most rats in about 4 weeks; at a concentration of 0.5 per cent it is less toxic. With either dose level the adrenals enlarge progressively. The adrenal weights at 28 days are 23.4 and 30.1 mg. per cent, respectively, as compared to 15 mg. per cent for untreated

TABLE 3. EFFECT OF HYPERTHYROIDISM, INDUCED BY FEEDING THYROID POWDER IN FOOD, ON ADRENAL, THYMUS AND THYROID WEIGHTS IN ADULT MALE RATS

Treatment	No. rats	Wt, grams	Adrenal wt.		Thymus wt.		Thyroid wt.	
			mg.	mg./100 g.	mg.	mg./100 g.	mg.	mg./100 g
None	4	242	36.4	15.0	649	268	21.5	8.9
*0.5 per cent thyroid powder in food**								
7 days	2	157	37.0	23.6	550	350	12.3	7.9
14 days	2	161	36.3	22.5	535	332	10.3	6.4
28 days	2	193	45.1	23.4	303	157	11.1	5.8
56 days	1	252	64.0	25.4	600	238	3.0	1.2
1 per cent thyroid powder in food†								
7 days	2	162	31.1	19.2	680	420	13.1	8.1
14 days	2	168	36.5	21.7	477	284	10.1	6.0
28 days	1	180	54.1	30.1	316	176	11.9	6.6

* 1 rat died during sixth week of the experiment.
† 3 rats died during fourth week of the experiment.

animals (Table 3). The thymus initially enlarges and subsequently atrophies. The thyroid gland becomes steadily smaller. The histological sections show that the adrenal enlargement is the result of broadening of the cortex, particularly of the zona fasciculata (cf. Figs. 2 and 16).

Cytochemically the results are different in the 2 groups. In the group receiving 0.5 per cent thyroid powder in the diet, the sudano-

philic zone of the glomerulosa initially becomes narrower than normal but recovers by the end of 2 months. During the reduction in sudanophilia the other ketosteroid reactions become weak or absent, particularly fluorescence and the Schiff reaction (Fig. 14), but they subsequently return to normal. The fasciculata, however, broadens and at 2 weeks the sudanophilic material, the Schiff-positive material and the autofluorescence appear slightly subnormal in quantity (Fig. 14), and the cells of the outer fasciculata are often vacuolated. The birefringence, on the other hand, becomes accentuated (Fig. 15), much as occurs during the adaptation syndrome resulting from inanition (Deane and Shaw, 1947, Figs. 9 and 10). By the end of 1 and 2 months, the fasciculata contains irregular regions in which the lipids are virtually exhausted, although other areas have normal contents. Moreover, a number of highly fatty cells containing abnormally large fat droplets characteristic of degeneration appear in the inner fasciculata and reticularis (Figs. 1 and 16). The number of these cells far exceeds that ever seen in the adrenals of normal animals.

With a 1.0 per cent level of thyroid powder in the diet, the lipid zone of the glomerulosa similarly becomes progressively thinner and contains less than normal ketosteroid activity. The broadened fasciculata at first appears to contain more Schiff reactive, birefringent and sudanophilic lipid than normal (Fig. 17). By the end of a month, however, the fasciculata is irregular in appearance, much as with 2 months' treatment with the smaller dose, and extremely fatty cells are scattered throughout the inner fasciculata. Moreover, the reticular formation of the cords of cells of the inner part of the cortex is accentuated.

DISCUSSION

In the following discussion a distinction will be drawn between atrophy and exhaustion of the 2 secretory zones of the cortex. In both conditions there is an ultimate decrease in the lipid content. However, in atrophy the zone shrinks and the birefringent particles become predominantly coarse in character, as is characteristic of inactivity (Weaver and Nelson, 1943). Exhaustion, on the other hand, follows a stage in which the zone displays an increase in ketosteroids, with the birefringent particles being fine, as is characteristic of active secretion. Atrophy indicates lack of secretion by the zone, whereas exhaustion reveals hypersecretion, with an inability to form as much hormone as is needed.

In hypothyroidism, whether caused by thyroidectomy or by treatment with thiouracil, the fasciculata of the adrenal cortex of rats atrophies and displays a gradual decline in sudanophilia and other ketosteroid reactions. Such atrophy has been observed by Rokhlina (1940), Leblond and Hoff (1944) and Glock (1945), although some other workers have failed to detect it (Williams et al., 1944; Leathem, 1946). Since the decline of lipid is progressive and not preceded by a

stage in which there is an increase in quantity, as happens in the adaptation syndrome, it may properly be considered to reflect the general decrease in metabolic and synthetic activities associated with hypothyroidism. This decline presumably reflects a reduced output of adrenotropin from the pituitary. Such a conclusion is more reasonable in light of current endocrinological knowledge than the hypothesis long held by Marine (1935) that an antagonism exists between thyroid and adrenal cortical functions. As another sign of general inactivity, the thymus undergoes slow atrophy.

Although the data are as yet inadequate, individual rats, after prolonged treatment with thiouracil, often possess adrenals of normal size which have a normal amount of steroid in the fasciculata (Figs. 10 and 11). Such recovery from cortical atrophy was noted earlier by Baumann and Marine (1945). This condition may represent the recovery of the pituitary-adrenal apparatus because of transient stresses such as infection, but no data are available in our records to make this more than a suggestion.

Although the fasciculata shrinks and becomes deficient in lipids after either thyroid removal or thyroid inhibition by thiouracil, some differences exist in the response of the zona glomerulosa to these two treatments. After thyroidectomy, the glomerulosa retains its ketosteroids as indicated by the cytochemical indices—indeed, the quantity of steroid appears to be increased (Fig. 5). This increase may be a response to the slightly decreased calcium level in the blood in rats following the unavoidable parathyroidectomy (Tweedy and Chandler, 1929). After treatment with thiouracil, however, the glomerulosa becomes exhausted, more completely in the rats receiving about 75 mg. daily than in those receiving about 25 mg. Perhaps this exhaustion can be explained by a fall in blood sodium resulting from decreased reabsorption because of casts present in the kidney tubules. The sulfa-containing compounds readily precipitate in the urine, and

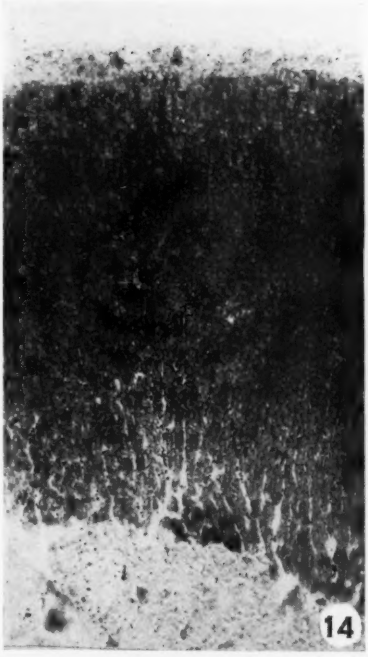
Explanation of figures on Plate 3

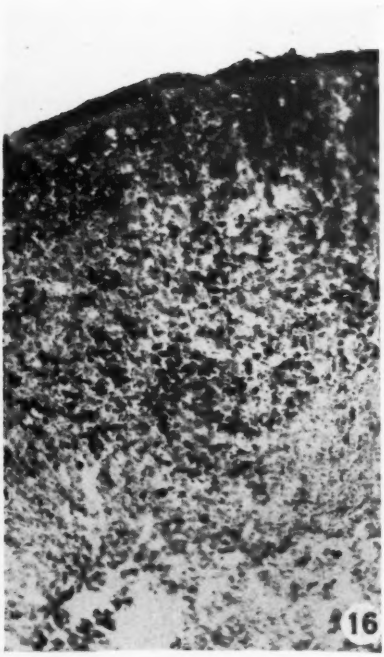
FIG. 14. Adrenal cortex of rat receiving 0.5 per cent thyroid powder in diet for 14 days. Schiff plasmal reaction. The fasciculata is wider than that of controls (Figs. 2 and 3). The plasmal reaction is almost negative in the zona glomerulosa, moderately intense in the fasciculata, and weak in the reticularis.

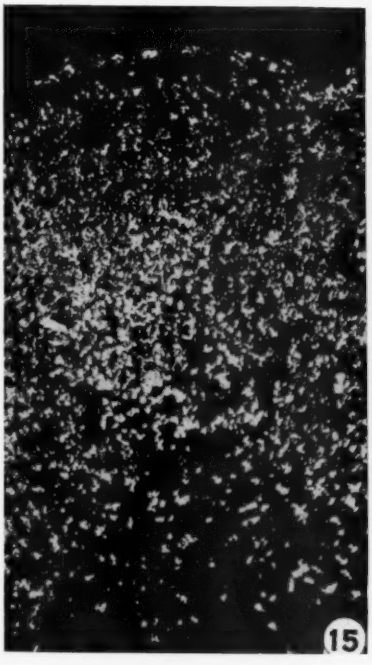
FIG. 15. Adrenal cortex of rat receiving 0.5 per cent thyroid powder in diet for 14 days. Birefringence pattern. The fasciculata is hypertrophied. Birefringent particles occur in the zona glomerulosa and throughout the fasciculata. Many are fine, characteristic of active secretion.

FIG. 16. Adrenal cortex of rat receiving 0.5 per cent thyroid powder in diet for 56 days. Sudan IV and hematoxylin. Sudanophilic lipids occur in the zona glomerulosa and fasciculata. In the inner fasciculata there occur many intensely fatty cells such as are illustrated in Fig. 1. Although similar cells sometimes occur in normal adrenals, they are infrequent and are generally limited to the reticularis.

FIG. 17. Adrenal cortex of a rat receiving 1 per cent thyroid powder in diet for 28 days. Schiff reaction. As in Fig. 14, the glomerulosa is blank. The outer part of the fasciculata contains considerable Schiff-positive material. Many excessively fatty cells occur at the inner border of this region.







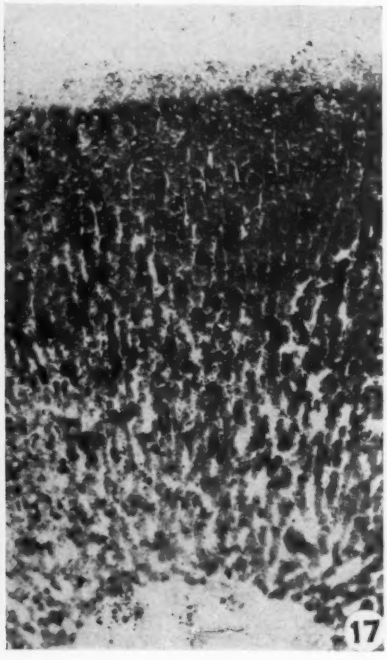


PLATE 3

a rise in serum chlorides with thiouracil treatment has been previously reported by Astwood (1945). That the glomerulosa becomes stimulated and eventually exhausted in conditions of reduced blood sodium has been indicated by other experiments (Knowlton et al., 1947; Deane and Shaw, unpublished; Olson and Deane, unpublished). Apparently, however, in rats receiving only 25 mg. thiouracil daily there is an eventual acclimatization to the treatment and a recovery of a normal salt balance. The differences of reaction of the glomerulosa in the two types of hypothyroidism may thus be assumed to be only differences of degree, with the stimulus in thyroidectomy being mild, while that with thiouracil treatment is so severe as to exhaust the zone.

Conversely, hyperthyroidism results in a stimulation of the zona fasciculata, as reflected by an increase in zone width and first an augmentation of and then a decrease in the quantity of ketosteroids. Furthermore, signs of cell degeneration become unusually prevalent in the inner zones (Figs. 1 and 16). This apparent stimulation in activity of the fasciculata in hyperthyroidism has been noted by Deanesly (1931) and Rokhlina (1940) and resembles that occurring in the adaptation syndrome (Deane and Shaw, 1947). It may thus be assumed to depend on an increased production of the adrenotropic hormone of the anterior pituitary. The changes in thymus size also suggest this, since, although there is an early increase in size probably referable to the increased metabolic rate, the subsequent extreme atrophy suggests a high level of secretion of the 11-oxy-corticosteroids, which are believed to be produced in the fasciculata (Deane and Shaw, 1947).

The zona glomerulosa also shows a progressive exhaustion with thyroid treatment. The only available explanation for this is that, since polyuria exists with thyroid treatment (Marine, 1935), there is probably an excess loss of sodium. Calcium is also excreted excessively with such treatment (Aub et al., 1929), with the result that there may be a compensatory rise in blood potassium and fall in blood sodium. Such a deduction is supported by the results of Lowenstein and Zwemer (1943), who found that potassium is more toxic in conditions of hyperthyroidism than in normal animals.

SUMMARY AND CONCLUSIONS

In hypothyroidism, induced either by thyroidectomy or by 2-thiouracil, the zona fasciculata of the adrenal cortex atrophies and its ketosteroid content is reduced. The zona glomerulosa is apparently more active than normal in both conditions, containing an augmented amount of steroid after thyroidectomy but becoming at least temporarily exhausted with thiouracil treatment.

In hyperthyroidism, induced by feeding thyroid powder, the zona fasciculata of the cortex hypertrophies. Its ketosteroid content becomes at first augmented and subsequently depleted. Abundant signs

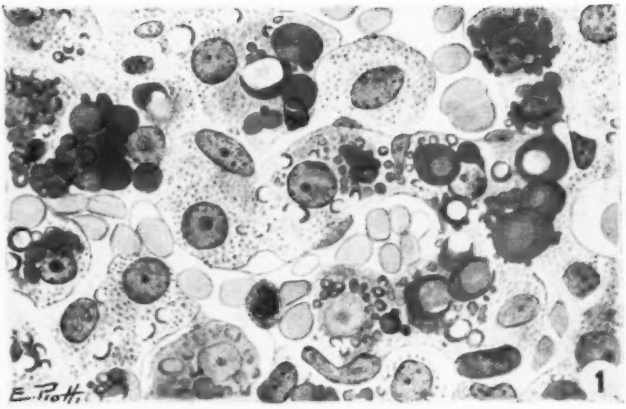


FIG. 1. Camera lucida drawing of cells in the inner fasciculata of the adrenal cortex of an animal receiving 0.5 per cent thyroid powder in the diet for a period of 56 days. Frozen section, 15 μ, stained with sudan IV and Harris' hematoxylin. The cords of cells run from left to right. Many of the cells contain tremendous fat droplets. In the others, smaller and more typical lipid droplets occur. Spherical gray-orange mitochondria may be seen in the less fatty cells. (Low-power photomicrograph of the same cortex, Figure 16.) ×90 objective, ×7 ocular; ×1180 at the plane of the drawing.

of cell death appear in the inner fasciculata. The zona glomerulosa becomes exhausted of ketosteroids.

The changes in the zona fasciculata are attributed to a lowered output of adrenotropin in hypothyroidism and an increased output in hyperthyroidism. The increase in activity of the zona glomerulosa in the several conditions is tentatively attributed to alterations in salt balance resulting from the different treatments.

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THE ADRENAL AND EARLY HEPATIC REGENERATION

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WITHIN twenty-four hours after removing 65 to 70 per cent of the liver of rats, a marked increase in the weight of the remnant takes place which is due largely to an increase in its fat and water content (Brues et al., 1936; Bogetti, 1939; and Collip et al., 1935). Fasting augments the deposition of fat, but impairs the weight increase (Collip et al., 1935). Adrenalectomy greatly decreases the accumulation of fat (Collip et al., 1935; MacKay, Eaton and Carne, 1938), but an effect on the weight increase has not been reported.

During an investigation of the influence of various endocrine glands on fat mobilization in fasted, partially hepatectomized rats, it was noted that the liver remnant in adrenalectomized animals was not only less fatty but also much smaller than that in controls (Berman, 1946). The difference in size appeared larger than could be accounted for merely by decreased fat deposition. The present communication reports our findings on the effect of adrenalectomy and adrenal hormones on early hepatic regeneration.

EXPERIMENTAL

Three experiments were performed. In the first, we examined the effect of adrenalectomy on the rate of weight increase and fat deposition in the liver remnant during the first twenty-four hours after partial hepatectomy in the fasted rat. At first immature rats were employed, but since they provided too little tissue for chemical analysis, adults were used in subsequent experiments. The second experiment was performed to confirm in adults, the effect of adrenalectomy since hepatic regeneration varies inversely with age (Norris, Blanchard and Povolny, 1942). The third experiment demonstrated the relative abilities of cortical extract and desoxycorticosterone acetate (D.C.A.) to substitute for the adrenal in promoting hepatic regeneration.

Experiment 1: The effect of adrenalectomy on the rate of early hepatic regeneration

Two groups of male, piebald rats weighing 90 to 110 gm. (average 105 gm.) were employed. The first group of 28 animals was partially

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hepatectomized, and the second group of 40, adrenalectomized and partially hepatectomized. All animals were individually caged, fasted and given 1% NaCl to drink. Seven of each group were sacrificed 6, 12 and 18 hours post-operatively and the remaining 6 and 12, were killed at 24 hours. The dry weights and fat concentration of liver lobes 1 and 2 (median and left lateral), removed at operation, and of the remnant at autopsy were determined. All material soluble in Bloor's solvent (1 part ethyl ether, 3 parts ethyl alcohol) was considered fat. The dry weight of the liver remnant at the time of operation, was calculated from the weight of liver removed, considering that lobes 1 and 2 comprised $70.5 \pm 0.9\%$ of the total liver weight in this strain and size of rat. Results are summarized in Table I.

A statistically significant difference between the weights of liver remnants in experimental and control rats was apparent twelve hours

TABLE I. EFFECT OF ADRENALECTOMY ON INCREASE IN LIVER REMNANT WEIGHT AFTER PARTIAL HEPATECTOMY AND FASTING

Group	Treatment	Hours after operation	Final body weight, gm.	Dry liver weight		Fat concentration	
				Remnant weight at operation (calculated) mg.[1]	Remnant weight at autopsy mg.[1]	At operation gm. fat per 100 gm. dry liver[1]	At autopsy gm. fat per 100 gm. dry liver[1]
1a	Control	6	94	490±26	485±26	20±1.6	33±3.6
2a	Adrenalectomized	6	100	464±24	478±11	24±2.2	24±2.6
1b	Control	12	85	415±26	467±26	24±1.8	35±1.4
2b	Adrenalectomized	12	98	405±12	380±20	24±1.6	29±1.3
1c	Control	18	96	458±47	499±21	22±1.3	43±5.0
2c	Adrenalectomized	18	93	381±6	371±18	20±0.9	35±3.1
1d	Control	24	87	462±12	529±33	24±2.7	39±1.9
2d	Adrenalectomized	24	95	437±11	399±33	21±1.5	30±1.8

[1] With standard errors.

after partial hepatectomy, while a difference in fat concentration appeared within six hours. The concentration of fat in the livers of adrenalectomized rats did increase, however, reaching a maximum at 18 hours as with the controls. The final weights of the liver remnants were consistently lower than the calculated weights at operation in the adrenalectomized rats, and consistently higher in the controls. The differences in remnant weights were much greater than could be accounted for by differences in fat concentration, hence, the inhibiting effect of adrenalectomy on early hepatic regeneration could not be due merely to a decreased ability to mobilize fat.

Experiment 2: Confirmation of inhibition of early hepatic regeneration in adrenalectomized adult rats

Three male albino rats weighing 195 to 215 gm. were partially hepatectomized, three were adrenalectomized and partially hepatectomized and six were kept as fasted controls to determine the normal weight of that portion of the liver equivalent to the remnant in par-

tially hepatectomized animals. In the previous experiment the "expected" weight of the liver remnant was calculated, as described, from the weight of the portion removed at operation. The resultant figure did not take into consideration the decrease in weight which normally accompanies fasting. Thus the values obtained were probably somewhat too high. As before, the rats were caged separately, given 1% NaCl to drink and sacrificed 24 hours post-operatively. The liver remnants, or the equivalent portions in unoperated rats, weighed 3.024 ± 0.217 gm., 1.844 ± 0.025 gm. and 1.830 ± 0.141 gm. in the partially hepatectomized, adrenalectomized and partially hepatectomized, and control groups respectively. Thus adrenalectomy completely prevented the increase in liver remnant weight normally seen 24 hours after partial hepatectomy in the fasted rat.

Experiment 3: The effect of cortical extract and desoxycorticosterone acetate on early hepatic regeneration in adrenalectomized rats*

In this experiment four groups of 10 male albino rats weighing 210 to 240 gm. (average 225 to 230 gm.) were employed. All were partially hepatectomized and those of the first three groups were also adrenalectomized. Rats of group 2 were injected with a total of 20 mg. of an oily solution of desoxycorticosterone acetate (D.C.A.) in four injections, and those of the third group with a total of 2 cc. of adrenal cortical extract* (30 dog units/cc.) in five injections. Each was separately caged and given 1% NaCl to drink. A synthetic, protein-free diet was given from the time of operation in order to minimize the formation of "acute fatty livers" (Collip et al., 1935) and the utilization of body food stores for maintenance metabolism. The diet was composed of: corn starch, 89%; vegetable fat, 1%; minerals (Steenbock and Nelson, 1923), 4%; cod liver oil, 1%; cellu-flour, 1%; sodium chloride, 4%; choline chloride and B-vitamin supplements (Hay, Prado and Selye, in press).

The wet and dry weights of the livers removed at operation (lobes 1 and 2) and of the remnants at autopsy were determined, as well as the total fat per 100 gm. of dry tissue and the total nitrogen per 100 gm. of fat-free dry tissue. Fat was estimated as in Experiment 1. Estimations of tissue nitrogen were made by an adaptation to the photoelectric colorimeter of Folin's direct nesslerization method (Folin, 1934). The values obtained were employed to calculate the percentage of fat and protein in wet tissue before and after operation, and the total protein content of the experimental remnants as compared with that calculated to have been present at the time of operation. The remnant weight was calculated as 25.2% instead of 29.5% of the total original liver weight since lobe 5 (caudate), which amounted to $14.6 \pm 1.3\%$ of the remnant was sectioned to observe mitoses. The protein content was assumed to be 6.25 times the total nitrogen content.

* Connaught Laboratories, Toronto, Ontario.

The data obtained are summarized in Table II. From a comparison of the average final body weights it will be noted that only the merely hepatectomized rats (group 4) lost a significant amount of weight in 24 hours. This tendency of adrenalectomized rats to maintain their operative body weights was apparent also in Experiment 1 in which the animals were fasted. The numbers of animals indicated in each group are those surviving until autopsy.

No difference was seen in the weight or composition of liver removed at operation. Presumably, therefore, all groups were similar at this time. At autopsy, the water content was increased and protein content decreased from pre-operative levels to approximately the same extent in all groups. There occurred also a marked increase in the amount of fat per 100 gm. of dry tissue which was least in the controls, and most in D.C.A.-treated animals. It is possible that the differences in fat concentration in animals of different groups may have been due in part to differences in food intake. All rats ate some of the food provided but the amount eaten was not measured. In order to estimate the amount of regeneration, the operative weight of the dry liver remnant was calculated, as in Experiment 1, from the weight of liver removed. It amounted to an average of 624 ± 3 mg. for all 35 rats. The dry weight of the remnant at autopsy in merely adrenalectomized rats was not significantly increased over this operative weight, while that in all other groups was increased. The increase could not be accounted for by changes in the water content of the tissue. Changes in fat concentration probably accounted for the increase seen in the D.C.A.-treated adrenalectomized rats, but not that obtained in cortical extract-treated animals. On the basis of the nitrogen content of the tissue an estimate was made of the total amount of protein in the liver remnant at autopsy. The values obtained are shown in the last column of Table II. The liver left at the time of operation was calculated to contain 407 ± 10 mg. of protein. Significantly increased amounts of protein were deposited in the liver remnants of normal rats, and adrenalectomized, cortical-extract-treated rats, but not of merely adrenalectomized animals or those treated with D.C.A. This difference in protein mobilization was accompanied by a difference in the average number of mitotic figures present. The number of mitotic figures was counted in five fields, taken at random, in a microscopic section of lobe 5 (caudate) from each animal, at a magnification of 400. The average number per field was 0.17, 0.20, 0.71 and 1.1 respectively for the four groups.

Thus, adrenalectomy impaired the deposition of protein, and the multiplication of liver cells 24 hours after partial hepatectomy. Administration of cortical extract, but not of D.C.A., replaced these functions of the adrenal.

Failure of D.C.A. to stimulate early hepatic regeneration in adrenalectomized rats was unexpected since D.C.A. causes an increase in liver weight in normal animals. It is possible that the failure to

TABLE II. EFFECT OF D.C.A. AND CORTIN ON EARLY HEPATIC REGENERATION IN ADRENALECTOMIZED RATS

Group	Treatment	Final body weight, gm.	No. rats	Liver												
				Removed at operation						Remnant at autopsy						
				Dry weight, gm.	Gm. fat in 100 gm. dry liver[1]	Gm. nitrogen per 100 gm. dry fat-free liver[1]	% water[1]	% fat in fresh liver[1]	% protein in fresh liver[1]	Dry weight mg.[1]	Gm. fat in 100 gm. dry liver[1]	Gm. nitrogen in 100 gm. dry fat-free liver[1]	% water[1]	% fat in fresh liver[1]	% protein in fresh liver[1]	Mg. protein in whole remnant[1]
1	Adrenalectomized	213 ±4	9	1.654	13.2 ±.4	12.2 ±.3	70.7 ±.4	3.9 ±.15	19.3 ±.5	642 ±34	27.9 ±2.0	13.2 ±.2	76.4 ±.8	6.6 ±.5	14.1 ±.7	376 ±28
2	Adrenalectomized D.C.A.	227 ±8	9	1.637	14.4 ±.4	12.2 ±.4	71.2 ±.2	4.2 ±.15	18.8 ±.6	751 ±37	31.6 ±2.2	13.1 ±.3	74.9 ±.6	8.0 ±.6	14.1 ±.7	410 ±22
3	Adrenalectomized cortical abstract	233 ±7	8	1.628	14.5 ±.5	12.4 ±.2	70.7 ±.2	4.4 ±.16	19.6 ±.4	822 ±36	29.8 ±1.4	13.6 ±.2	74.9 ±.8	7.2 ±.3	14.4 ±.7	488 ±21
4	Controls	207 ±4	9	1.664	15.1 ±.5	11.8 ±.2	71.0 ±.2	4.2 ±.14	18.3 ±.4	874 ±35	21.5 ±1.2	11.9 ±.3	75.4 ±.4	5.3 ±.3	13.8 ±.3	494 ±24

[1] Standard error.

observe a significant increase in the mitotic rate was due to the inability of tissue to respond rapidly to D.C.A. We have observed repeatedly that in periods of 5 to 10 days after partial hepatectomy, D.C.A. markedly stimulates hepatic growth in normal animals. In one experiment, for example, 14 female rats, 90 to 100 gm. in weight were partially hepatectomized and 7 were injected post-operatively twice daily for 5 days with 5 mg. of D.C.A in oil solution. The liver remnant weighed 4.9 ∓0.2 g. in the treated rats and 3.9 ∓0.2 g. in the controls, with final body weights of 100 and 98 gm. respectively. In another experiment, 20 female rats, 40 to 60 gm. in weight were treated for 10 days with the same dose as used above. The liver remnant weighed 4.9 ∓0.4 g. in the treated and 3.4 ∓0.2 in the controls, with final body weights of 75 and 79 g. These liver remnants were analyzed for their water, fat and nitrogen content. The protein content was calculated from the nitrogen as indicated above. The livers of D.C.A. treated rats contained significantly more water (75.3 ∓0.2% *vs.* 73.5 ∓0.3%). No difference was apparent in the concentration of protein and fat expressed per unit of wet tissue. Thus, under these circumstances D.C.A. did promote hepatic regeneration.

DISCUSSION

The role of the adrenal cortical hormones both in protein degradation and in adaptation has long been recognized. The present experiments suggest that these hormones might also be involved in protein synthesis and cellular regeneration, but more data is needed before this subject can be adequately discussed.

With a diluted solution of adrenal extract, growth was allegedly stimulated in the unicellular organism (Frontonia) (Sokoloff, 1929), but in tissue culture both D.C.A. and cortical extract have been found to depress mitotic activity (Haam and Cappel, 1940; Möllendorff, 1942). The concentrations employed may have been too large since abnormal mitoses were observed with these and other steroids which seemed to parallel the degree of unsaturation of the compound rather than any hormonal activity (Möllendorff, 1942). In regard to wound healing, the local application of adrenal pulp was reported to increase the production of granulation tissue (Voronoff and Bostrich, 1918; Aievoli, 1923) while experiments with partial and complete adrenalectomy have led to contradictory results (Santorsola, 1931; Kosdoba, 1934). In our third experiment, sections of sutured wounds from each animal were examined. There was some suggestion that movement of epithelial cells over the wound surface was impaired in untreated and D.C.A.-treated adrenalectomized rats as compared with the controls and the cortical-extract-treated adrenalectomized animals. However, neither these cells nor the fibroblasts were in active proliferation.

The depression of hepatic regeneration by adrenalectomy was not

due to differences in food intake since it was seen in fasted as well as in fed rats. It may be related, however, to the amounts of materials brought to the liver from depots. Fasted adrenalectomized animals lost significantly less weight than did fasted controls. Changes in the amount of depot protein were not estimated, but it is known that the thymus, from which protein is rapidly available (White and Dougherty, 1944) is under adrenal control (Selye, 1936). In regard to fat depots, attention was paid in the first experiment to the relative amounts of retroperitoneal fat in animals of the various groups. Although the amount present was roughly inversely proportional to the degree of fatty infiltration in the liver, the subject should be studied more quantitatively before making any definite statement.

CONCLUSIONS

Adrenalectomy decreases the deposition of fat, inhibits the deposition of protein and prevents the increase in size and cell number normally seen in the liver remnant of rats 24 hours after partial hepatectomy. Adrenal-cortical-extract, unlike desoxycorticosterone acetate (D.C.A.) substitutes for these functions of the adrenal. However, D.C.A. can increase the rate of hepatic regeneration in normal rats when measurements are made five days post-operatively.

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NOTES AND COMMENTS

SPECIES DIFFERENCE IN THE CORTICAL HORMONE REQUIREMENT OF ADRENALECTOMIZED DOGS AND CATS

A NUMBER of years ago some of my colleagues and I presented data on the cortical extract requirement of the adrenalectomized dog and cat (Cleghorn, McHenry, McVicar, and Overend, 1937). The assays conducted on these animals disclosed the fact that cats required about ten times as much extract per Kg. body weight as the dogs. Data on three cats were given; three others were done later with similar results, the requirements being 0.38 to 0.82 cc. per Kg. with an average of 0.63 cc. The minimal requirement of three dogs done at the same time was 0.036 and 0.055 and 0.090 cc. per Kg. The diet used for the cats consisted of canned salmon, liver and evaporated milk. This food was well taken. Analysis showed it to contain by dry weight NaCl 3.82 per cent, K 1.45 grams per cent. The dogs' diet, a mixture of Purina Dog Chow and meat, contained NaCl 2.6 grams per cent; K 1 to 1.59 grams per cent. In spite of the higher sodium intake, therefore, the cats required many times as much extract per kilo body weight. Whole beef gland extract prepared by the Connaught Laboratories was used. The assay method of Harrop, Pfiffner, Weinstein and Swingle, 1932, was employed, viz. determination of the minimal amount of extract necessary per day to maintain animals' health and weight and prevent a 100% increase in blood urea or N.P.N.

Subsequent to the above studies we have investigated the Desoxycorticosterone Acetate requirement of adrenalectomized dogs (Cleghorn, Fowler, Wenzel and Clarke, 1941) but have not made similar determinations on cats. This should be done.

It is well known to investigators who have had experience with both adrenalectomized dogs and cats that the former seldom develop hypoglycemia, the latter frequently. This suggests inherent differences in metabolism which bears investigation. Careful assays of the cortical hormone requirement of these, and other, species by other technics than the now outdated method employed by us ten years ago is indicated. The obvious interpretation of our findings is that cats require more cortical hormone of the type affecting electrolyte metabolism than dogs. However, the cats' diet was higher in protein than the dogs' and this may have favored an earlier rise in blood N.P.N. It may well be that cats require much more of the C^{11} oxygenated carbohydrate active steroids than dogs under these conditions for purposes of gluconeogenesis so that the greater hormone requirement be for the carbohydrate active rather than for the electrolyte active properties of the extract. Investigations would well repay the effort by the settling of this point and possibly by the disclosure of significant differences in me-

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tabolism in the two species. For what it is worth this note is published since I do not expect to have the opportunity to follow up this problem.

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MITOTIC AND WOUND HEALING ACTIVITIES OF THE CORNEAL EPITHELIUM IN THIOURACIL TREATED AND THYROIDECTOMIZED RATS

THE CORNEAL epithelium of the rat is a test-object excellently suited for investigations on the factors influencing mitosis and post-traumatic cell movement. The present paper is concerned with the effect of thyroid deficiency on these cellular activities.

MATERIAL AND METHODS

Sixty-two male albino rats of between 100 and 200 gm. were used in the experiment. Thiouracil[1] was added to the drinking water in a concentration of 0.1 per cent for at least six weeks prior to the experiments. Surgical thyroidectomy was carried out in a group of 10 rats of about 100 gm. weight. Only the 6 rats, which showed marked growth retardation were used in the experiment. No thyroid tissue could be found in these animals at autopsy except in one rat, in which a tiny remnant of the thyroid gland could be detected.

The method of assaying mitotic activity has been published elsewhere in detail (Buschke, Friedenwald and Fleischmann, 1943). Counts are made on flat preparations of a meridional strip of the cornea. However, mitotic activity cannot be gaged solely by the number of mitoses present at a single moment, since that number is affected both by the rate at which cells enter mitosis and by the rate at which cells pass through the mitotic cycle. For the purpose of analyzing the rate of mitosis it is necessary to employ a drug which inhibits mitosis at a given point. Colchicine has been proved to be a useful tool as its predominant effect is to arrest mitosis in metaphase without influencing the rate at which cells enter mitosis. This has been shown convincingly in studies on tissue cultures (Bucher, 1939). We have found the effect of colchicine on the corneal epithelium to be of the same kind. Hence the number of cells in the corneal strip observed in mitosis at a fixed time after administration of colchicine enables us to estimate the rate at which cells enter mitosis (Buschke, Friedenwald and Fleischmann, 1943).

Post-traumatic cell movements were studied by observing the closing of

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[1] Thiouracil (Deracil) powder was kindly supplied by Lederle Laboratories, Inc., Pearl River, N. Y.

minute epithelial defects produced by needle pricks of the corneal surface. (Friedenwald and Buschke, 1944a). The defects produced measure about 30 μ in diameter and are covered three hours after injury. This process can be best studied on flat preparations of the cornea.

RESULTS

Mitosis counts in a meridional strip 0.11 mm. in width ranged from 62 to 119 with an average of 92 in control rats. In thyroidectomized rats the range was from 72 to 121 with an average of 100. In another series of experiments 5 mg. of colchicine per kg. bodyweight was given intramuscularly 4 hours before sacrificing the animals. In this series the range was from 260 to 431 with an average of 393 in the normal and from 120 to 237 with an average of 168 in the thiouracil treated rats. The averages and standard deviations of these counts are listed in Table I. It is evident that only in the experiments with colchicine is the difference between controls and thiouracil treated rats statistically significant. In the six animals in whom the thyroid

TABLE I. CORNEAL MITOTIC COUNTS

	Without	With
	Colchicine	
Controls	92 ± 35	393 ± 59
Thiouracil Treated	100 ± 17	168 ± 42
Thyroidectomized		228 ± 41

had been removed successfully the colchicine technique was applied. The mitotic counts varied from 182 to 290 with an average of 228. The difference between the counts in the control and in the thyroidectomized rats is statistically significant. There is no significant difference between the thyroidectomized and the thiouracil treated rats.

The corneal strips of the various groups showed no marked differences in length. It is well known that the growth of the eyeball in the rat is little affected by thyroid deficiency (Hammett, 1927).

Small epithelial wounds were produced by pin-pricks in the corneal epithelium of six thiouracil treated rats and six controls. The eyes were enucleated and fixed in formol-alcohol three hours after injury. In all eyes the small defects were completely covered by epithelium.

DISCUSSION

Partial or total ablation of the thyroid gland in experimental animals results in a definite retardation of wound healing (Arey, 1936). Kosdoba (1934) attributes this retardation to the lack of thyroid hormone, which influences the connective tissue and blood vascular system. He demonstrated this on experimental wounds involving the skin, the subcutaneous tissue and the muscle. It is therefore of interest, that no retardation is observed in wounds of the epithelium of the avascular cornea. The closure of these minute wounds is accomplished by movement of epithelial cells only. It was shown first by Smelser (1945, 1947) that marked thyroid deficiency does not affect the epithelization of corneal burns. The difference in the rate of

cell migration between the thyroidectomized rats and their littermate controls was insignificant. Our observations confirm these findings. The wounds in our experiments were smaller than those produced by Smelser.

Mitotic activity is much more sensitive to pharmacological interference than the healing of minute wounds (Friedenwald and Buschke, 1944b). The same seems to be true for hormonal interference. The mitotic rate as estimated from the colchicine experiments was reduced in the thiouracil-treated rats by more than 50 per cent, but the number of mitoses found in the counts without colchicine was not significantly reduced. This discrepancy becomes understandable, if we assume that the mitotic cycle is slowed down in the same proportion as the entrance into mitosis. In the colchicine experiments we measure the rate of entrance into mitosis. In the counts without colchicine we measure the number of cells actually undergoing mitosis without any reference to the speed of the mitotic cycle. By combining the results of the experiments with and without colchicine we come to the conclusion that both the mitotic cycle and the entrance into mitosis are slowed down in hypothyroidism.

In order to exclude the remote possibility that this effect was due to a direct action of thiouracil on the corneal epithelium, the colchicine experiments were repeated on surgically thyroidectomized rats. The results were in good agreement with the results obtained by treatment with thiouracil.

The results are in good agreement with the opinion of developmental physiologists that thyroid hormone stimulates mitotic activity in the epithelium (Schulze, 1930).

However Smelser (1947) using the colchicine technique failed to find a significant difference between the mitotic rates in the corneae of normal and thyroidectomized rats. We are unable to explain the discrepancy between Smelser's and our own experiments.

Reduction in speed of the mitotic cycle associated with a decline in the rate of entrance into mitosis is not a phenomenon specific for thyroid deficiency. Similar observations have been made on rats after removal of the superior cervical sympathetic ganglion (Friedenwald and Buschke, 1944b) and on vitamin A deficient rats (Friedenwald, Buschke and Morris, 1945).

SUMMARY

The post-traumatic cell movements in the healing of small wounds in the corneal epithelium are not delayed in thiouracil treated rats. With the use of the colchicine technique it was found that both the entrance into mitosis and the speed of the mitotic cycle is reduced in thiouracil treated and in thyroidectomized rats.

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From the Physiology Section, Medical Division, Army Chemical Center, Edgewood, Md., and the Department of Pediatrics, Johns Hopkins University, Baltimore, Md. Read before the Association for the Study of Internal Secretions, Atlantic City, N. J., June 7, 1947.

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THE FAILURE OF SPLENECTOMY TO INFLUENCE THE UTERINE RESPONSE TO ALPHA-ESTRADIOL IN CASTRATE AND PARTIALLY-HEPATECTOMIZED CASTRATE RATS[1,2]

A NUMBER of workers have demonstrated that intrasplenic implants or injections of steroidal hormones are much less effective than subcutaneously-administered materials. Since a large measure of the effectiveness was restored by transplantation of the spleen to subcutaneous sites, it has been generally concluded that the reduced effectiveness was due to hepatic inactivation of steroidal material entering the portal circulation from the spleen *in situ* (cf. Biskind and Mark, 1939; Segaloff, 1943).

The possibility that the spleen itself may be responsible for the inactivation process, failing in this function when transplanted, has been almost completely overlooked. This has been the case even though Zondek's early *in vitro* experiments (1934) indicated that splenic tissue decreased the activity of estrogen incubated with it (not confirmed by Heller, 1940). Moreover, Biskind and Mark (1939), in their discussion of the observed reduced effectiveness *in situ* of splenic implants of testosterone and estrone, commented on the marked histological changes occurring in transplanted splenic tissue, which might be indicative of impaired function.

In the light of these observations and the recent demonstration (Roberts and Szego, 1947) that the liver may possess an "activating" in addition to an "inactivating" function for the estrogens, it was thought advisable to reinvestigate the role of the spleen in the activity of the steroid hormones.

The results reported below, however, indicate that splenectomy has no significant effect on the uterine response to intravenously-administered alpha-estradiol, either in castrate or in partially-hepatectomized castrate rats.

Most of the procedures employed have been described in detail in a previous report (Roberts and Szego, 1947). Female rats of an inbred Sherman strain, about two months old and weighing 110 to 125 gm., were ovariectomized. Approximately two weeks later, these animals were subjected to various operative procedures, including splenectomy, partial hepatectomy,

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[2] Crystalline alpha-estradiol used in this study was obtained through the courtesy of Dr. E. Schwenk of the Schering Corporation.

and splenectomy plus partial hepatectomy combined. Control animals were sham-operated, during which procedure the viscera were exposed and manipulated for a short time. Sham operation was uniformly found to produce no significant effect on the uterine response to intravenously-injected estrogen. Partial hepatectomy consisted of removal of approximately two-thirds of the liver. Splenectomy was carried out after ligation of the pedicle. Immediately after operation, test solutions of alpha-estradiol in saline, or control saline solutions, were injected into the saphenous vein (cf. Roberts and Szego, 1947). Four hours later, the animals were sacrificed and the uteri

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TABLE I. THE INFLUENCE OF SPLENECTOMY, HEPATECTOMY, AND SPLENECTOMY-HEPATECTOMY ON THE IMBIBITION OF WATER BY THE UTERUS IN RESPONSE TO VARYING DOSES OF ALPHA-ESTRADIOL[1,2]

Dosage (micrograms alpha-estradiol per 100 gm. body wt.	Sham	Splenectomized	Hepatectomized	Hepatectomized-Splenectomized
0[3]		79.2 ± 0.7 (76.6 to 80.2) —17—		
0.125	81.8 ± 1.5 (79.5 to 85.8) —13—	81.2 ± 1.1 (79.7 to 83.8) —17—	—	—
0.25	82.4 ± 1.9 (79.8 to 85.4) —8—	81.9 ± 2.5 (79.2 to 86.5) —10—	80.3 ± 1.3 (76.0 to 82.8) —16—	80.0 ± 0.5 (79.2 to 80.9) —8—
0.5	—	—	80.0 ± 0.5 (79.2 to 80.7) —8—	80.6 ± 0.7 (79.7 to 81.5) —5—
1.0	83.8 ± 1.3 81.7 to 85.5) —6—	83.3 ± 0.5 82.4 to 84.0) —6—	80.1 ± 1.3 79.2 to 82.2) —11—	80.5 ± 0.5 (79.7 to 81.3) —6—
2.5	84.5 ± 0.7 (83.6 to 86.0) —17—	84.6 ± 1.0 (83.3 to 87.4) —14—	81.1 ± 1.0 (80.2 to 83.1) —6—	81.2 ± 0.4 (80.6 to 81.7) —9—

[1] All animals were autopsied 4 hours after intravenous injection of estrogen, administered immediately after operation.

[2] Mean ± standard deviation shown, followed by the range in parentheses and the number of rats.

[3] The results obtained in all groups injected with saline alone have been combined, since the operative procedures employed have been found to have no significant effect on these values.

removed and weighed in the wet and dry state. From this data the per cent uterine water could be determined.

Table I gives the results obtained in all groups of animals. Only the per cent water figures are listed, since this criterion has been found to be the most reliable uterine index of response to estrogen under a variety of experimental conditions. It may be noted, however, that the wet and dry weight

figures, expressed as mgm. uterine weight per 100 gm. body weight, followed an essentially similar progression in the present experiments.

It is apparent that the dosage-response curve to alpha-estradiol is unaffected by splenectomy in either normal or partially-hepatectomized, castrate rats. The reduction in uterine response immediately after partial hepatectomy has been previously reported (Roberts and Szego, 1947).

COMMENT

The present study reveals that the spleen has no apparent influence on the uterine response of the ovariectomized rat to intravenously-administered alpha-estradiol. This appears to be true in partially-hepatectomized rats as well as in animals with intact livers. Since splenectomy, which would seem to constitute a considerable stress, does not result in a decreased uterine response, it seems unlikely that the reduction observed immediately after partial hepatectomy is simply a response to a non-specific stress.

The results reported above, it should be emphasized, do not indicate that the spleen may not alter the activity of estrogen injected by other routes, particularly intrasplenically. They also do not constitute evidence either for or against the possibility of *in vivo* or *in vitro* destruction of estrogenic material placed in intimate contact with splenic tissue.

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ASSOCIATION NOTICE

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb & Sons Award of $1,000.00 was established in 1939, and was given first in 1940 to Dr. George W. Corner; 1941—Dr. Philip E. Smith; 1942—Dr. Fred C. Koch; 1943—no award was given; 1944—Dr. E. A. Doisy; 1945—Dr. E. C. Kendall; 1946—Dr. Carl G. Hartman; 1947—Drs. Carl F. and Gerty T. Cori. A special Committee of five members of the Association chooses an investigator or investigators in the United States or Canada for one of the best contributions to endocrinology.

THE CIBA AWARD

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than 35 years of age in the field of endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russel; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li. The work cited may be either in the field of pre-clinical or clinical endocrinology. The Award is for $1,200.00. If within 24 months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, the Award will be increased to $1,800.00.

THE AYERST, McKENNA & HARRISON FELLOWSHIP

The Ayerst, McKenna & Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin. The fellowship was founded in order to encourage investigation in the field of endocrinology rather than as an award for work done. The amount of the fellowship is $2,500.00 annually. The nominee must possess the degree of Doctor of Philosophy or Doctor of Medicine or their equivalent. It is suggested that no restriction be placed on age, but that preference be given to applicants who have recently completed the requirements for their Ph.D. or M.D. degree. The nominee must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals; submit a program of proposed study; indicate one or more institutions where the proposed program shall be carried out; submit statement of approval from the investigators with whom he proposes to conduct his research; serve full time if awarded a fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

Each member has the privilege of making one nomination for each Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions in endocrinology and by a bibliography of the nominee's most important publications, and reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma, and returned to him not later than March 15, 1948.